Clinical Trial Protocol

A phase II stratified trial to assess haploidentical T-depleted stem cell transplantation in patients with sickle cell disease with no available sibling donor

Sponsor's Protocol No:	T-Haplo for SCD
EudraCT No.:	2018-002652-33
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Status:	Final
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Sponsored by:	DFG Deutsche Forschungsgemeinschaft

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RESPONSIBILITIES

Sponsor

University Hospital of Regensburg, Franz-Josef-Strauss-Allee 11, 93053 Regensburg is sponsor in terms of §4 (24) AMG (German Drug Law) and additional §§.

Responsible for observance of the sponsor's trial-related duties and functions and Prinicple Investigator

30. JAN. 2020	Prof. Dr. Selim Corbacioglu	
Date	*Name*	*Signature*

Investigator

Herewith I confirm that I have read the study protocol carefully and declare my consent to it. I will treat and examine the patients in accordance with the study protocol, the national applicable laws, the international guidelines of good clinical practice (ICH-GCP) and the Declaration of Helsinki.

Date	*Name*	*Signature*

ADDRESSES

Sponsor

University Hospital Regensburg
Franz-Josef-Strauss-Allee 11
93053 Regensburg

Coordinating Investigator and Principle Investigator

Selim Corbacioglu, MD
Department of Pediatric Hematology, Oncology and SCT
University Hospital Regensburg
Franz-Josef-Strauß-Allee 11
93053 Regensburg
Phone: +49 (0)941 944-2101
Fax: +49 941 944-2068
Email: selim.corbacioglu@ukr.de

Deputy Investigator

Jürgen Föll, MD
Department of Pediatric Hematology, Oncology and SCT
University Hospital Regensburg
Franz-Josef-Strauß-Allee 11
93053 Regensburg
Phone: +49 (0)941 944-12051
Fax: +49 (0)941 944-2002
Email: juergen.foell@ukr.de

Datamanagement

Rainer Spachtholz
Department of Pediatric Hematology, Oncology and SCT
University Hospital Regensburg
Franz-Josef-Strauß-Allee 11
93053 Regensburg
Phone: +49 (0)941 944-12097
Fax: +49 (0)941 944-2068
Email: rainer.spachtholz@ukr.de

SAE-Management

Pharmacovigilance Safety Desk
coTrial Associates
University Hospital Regensburg
Franz-Josef-Strauss-Allee 11
93053 Regensburg
Phone.: +49 (0)941 944-4897
Fax: +49 (0)941 944-6772

Leading Ethics Committee

Universität Regensburg
Ethikkommission
93040 Regensburg
Phone: +49 (0)943-4370
Fax: +49 941 (0)943-5369

Competent Authority

Paul-Ehrlich- Institut
Federal Institute for Vaccine and Biomedinices
D-63225 Langen
Phone: +49 (0)6103 77-0
Fax: +49 6103 77-1234

Local Competent Authorities

Regierung von Oberfranken
Sachgebiet Pharmazie
95444 Bayreuth

Trial Statistician

Dr. rer. nat. René Schmidt
Institute of Biostatistics and Clinical Research
University of Münster
Schmeddingstr. 56
48149 Münster
Phone: 0251-83-58370
Email: rene.schmidt@ukmuenster.de

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ADV	Adenovirus
AE	Adverse event
aGVHD	Acute graft-versus-host disease
ALAT	Alanine aminotransaminase (GPT, glutamic pyruvic transaminase)
ALL	Acute lymphoblastic leukemia
AML	Acute myeloic leukemia
AMG	Arzneimittelgesetz (German Medicinal Products Law)
AP	Alkalic phosphatase
AR	Adverse reaction
ASAT	Aspartate aminotransferase (GOT, glutamic oxaloacetic transaminase)
ATG	Anti-thymocyte globulin
ATIII	Antithrombin III
BID	Bis in die (twice daily)
BM	Bone marrow
BfArM	Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte)
BS	Body surface
BW	Body weight
CBC	Complete blood count
CI	Continious infusion
cGVHD	Chronic graft-versus-host disease
CK	Creatine phosphokinase
CMV	Cytomegalovirus
CNS	Central nervous system
CR	Complete remission
CRF	Case report form
CRP	C-reactive protein
CRS	Cytokine release syndrome
CSF	Colony stimulating factor
CTC	Common toxicity criteria (for adverse events)
DFS	Disease-free survival
DLI	Donor lymphocyte infusion
DNA	Deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
EBMT	European Group for Blood and Marrow Transplantation
EBV	Epstein-Barr virus
ECHO	Echocardiography
EDTA	Ethylenediaminetetraacetic acid
EFS	Event-free survival
ESR	Erythrocyte sedimentation rate
FACS	Fluorescence activated cell sorting
G-CSF	Granulocyte colony stimulating factor
GGT	Gamma-glutamyl transpeptidase
GVHD	Graft-versus-host disease
Haplo	Haploidentical
HbsAG	Hepatitis B surface antigen

HCG	Human chorionic gonadotropin
HCT	Hematopoietic cell transplantation
HCV	Hepatitis C virus
HHCT	Haploidentical allogeneic hematopoietic cell transplantation
HHV-6	Human herpes virus 6
HIV	Human immunodeficiency virus
HLA	Human leucocyte antigen
HRQoL	Health related Quality of Life
HSV	Herpes simplex virus
HSCT	Hematopoietic stem cell transplantation
ICH-GCP	Guideline for Good Clinical Practice of the International Conference on Harmonization
ICSR	Individual case safety report
IEC	Independent ethics committee
IMP	Investigational medicinal product
IMPD	IMP dossier
INR	International normalized ratio
IRB	Institutional review board
ITT	Intention-to-treat
LDH	Lactate dehydrogenase
LKP	Principal Coordinating Investigator (‚Leiter der klinischen Prüfung' according to German drug law [AMG])
LVEF	Left ventricular ejection fraction
MMF	Mycophenolate mofetil
MRD	Minimal residual disease
MSD	Matched sibling donor
MUD	Matched unrelated donor
ORL	Otorhinolaryngology
OS	Overall survival
PB	Peripheral blood
PBMC	Peripheral blood mononuclear cell
PBSC	Peripheral blood stem cell
PBSCT	Peripheral blood stem cell transplantation
PCR	Polymerase chain reaction
PEI	Paul-Ehrlich Institute
PGF	Primary graft failure
PP	Per protocol
PRES	Posterior reversible encephalopathy syndrome
PTLD	Post-transplant lymphoproliferative disease
PTT	Partial prothrombin time
QD	quaque die (once daily)
RIC	Reduced intensity conditioning
RNA	Ribonucleic acid
SAE	Serious adverse event
SAR	Serious adverse reaction
SCD	Sickle cell disease
SGF	Secondary graft failure
SOS	Sinusoidal obstruction syndrome

SUSAR	Suspected unexpected serious adverse reaction
TBI	Total body irradiation
T cells	Thymus-derived withe blood cells
TCR	T-cell receptor
TNF	Tumor necrosis factor
TOF	Time-of-flight
TREC	T-cell receptor excision circle: circular, stable extrachromosomal DNA fragment generated during T-cell receptor diversification
Treg	T regulatory cell
TRM	Transplant-related mortality
TSH	Thyreoidea stimulating hormone
VOD	Veno-occlusive disease
VZV	Varicella zoster virus
ADV	Adenovirus

1. PROTOCOL SYNOPSIS

EudraCT-No **2018-002652-33**	**Sponsor's Protocol No** T-Haplo for SCD
Status, version and date of clinical trial protocol Final, V1.0, 30.01.2020	
Title of the clinical trial	A phase 2 trial to assess haploidentical α/ß T-depleted stem cell transplantation in patients with sickle cell disease with no available sibling donor (T-Haplo for SCD)
Sponsor	University Hospital of Regensburg
Total number of trial participants	212
Multicentre Trial	7 participating centres
Coordinating Investigator	Prof. Selim Corbacioglu
Study population	• Homozygous hemoglobin S disease or heterozygous hemoglobin SC or S 0/+ • Age 1yr to 35yrs Patients eligible for allogeneic stem cell transplantation. Reference Arm: HLA-identical sibling donor (MSD) as determined by high-resolution typing Experimental Arm: Haploidentical donor if MSD not available
Study duration	First patient 'in' to last patient 'out' (months): 72 months Duration of the entire trial (months): 84 months Recruitment period (months): 48 months Individual patient follow-up: up to 24 months
Targeted Cellular Composition:	**Experimental Arm:** Graft: • Viable $CD34^+CD45^+$ cells Target cell number ≥1 × 10^7/kg BW, percentage of viable cells ≥95% • TCR αβ/CD3Tcells Target cell number ≤5 × 10^4/kg BW **Note:** In order to reach the target cell number of ≥1 × 10^7 $CD34^+CD45^+$ cells/kg BW, a maximum cell number of 5 × 10^4 TCRαβ/CD3T cells/kg BW must not be exceeded. • $CD20^+$ cells Targeted Cell number ≤1 × 10^5/kg BW Additional IMP specifications concern TCRγδ, CD3+ and CD45+/WBC counts, CD45+ viability, hematocrit and result of visual control. Sterility will be tested but is not relevant for release Route of administration: IV **Reference Arm:** Matched sibling donor (MSD) Graft: Bone marrow (BM) Route of administration: IV

Background and Rationale	SCD represents a worldwide health problem and is a progressively debilitating multi-organ disease. At least 2% of the world population carry a hemoglobin S variant and cause over 80% of hemoglobin disorders which contribute to the equivalent of 3.4% of mortality in children aged under 5 years. The exact number of patients treated in many western countries is unknown but awareness is growing and patients registered at pediatric hematology centres are rising exponentially [1]. In children, stroke is the major complication of SCD and SCD is the most frequent cause of stroke in children. 30% develop silent strokes leading to cognitive impairment in children and an impaired quality of life [2] [3]. SCD is a major public health concern, recognized by the World Health Organization (WHO) and the United Nations (UN) as a global problem. From 1989 through 1993, an average of 75,000 hospitalizations due to SCD occurred in the United States, with approximately $475 million (http://www.cdc.gov/ncbddd/sicklecell/data.html) of health care related expenses. The mainstay of treatment is erythrocyte transfusions and symptomatic pain management. More than 90% of adults receive at least one transfusion in their lifetime. Indications for chronic transfusion most frequently relate to stroke prevention, given that patients with previous strokes have a high risk for recurrence [4]. However, discontinuation of prophylaxis is associated with stroke recurrence and increased mortality [5]. Hydroxycarbamide (HU) was shown to induce Hemoglobin (HbF) in SCD [6] and reduced the median incidence of painful crisis per year by 44% [7]. In children with known cerebrovascular disease, a benefit of HU for primary and secondary stroke prevention was assumed [8], but HU and transfusions have to be continued life-long with the downside of iron overload and the potentially long-term adverse effects of chemotherapy, in particular in children. Hyper-transfusion cannot stop the disease progression with 17% recurrent strokes and 31% new silent infarcts [9]. Despite adequate care, the OS remains more than 20 years behind age matched peers [10]. HSCT is currently the only curative option for SCD but less than 20% of SCD patients have a matched donor (MD) available [11]. So far, all curative approaches beyond a MSD HSCT at young age are non-satisfactory. With the lack of a suitable donor for the vast majority of patients, the major question of this trial is, if a haploidentical αß/CD19+ T cell depleted HSCT can be a valid alternative to a MSD HSCT. The main challenge in non-malignant diseases is to offer a safe and GVHD-free HSCT without rejection. This is a particular challenge in SCD patients suffering from systemic vasculopathy, a high risk of alloimmunization and rejection due to multiple transfusions. Surveys among SCD patients revealed that GVHD was an unacceptable complication (80%) and should be avoided at all cost [12, 13]. The main questions therefore are: Safety of a α/ß T cell depleted haploidentical HSCT; Incidence of acute and chronic GVHD; Rate of rejection; Immune reconstitution; Fertility and quality of life post-HSCT. The intention of this trial is to assess T-depleted haploidentical HSCT as a valid treatment option for SCD, in particular when a shorter time to transplant, an accelerated rate of engraftment and a lower rate of acute and chronic GVHD proofs to match a MSD HSCT. The benefit of this trial for the individual, suffering from this devastating disease, is the proof that a safe procedure can cure without delay and a low risk for morbidity and mortality. In addition, the estimated lifetime cost of medical care for SCD patients was calculated at 460.151$ per SCD patient [14], with chronic transfusions and iron chelation costs increase with approximated additional 40.000$/year. A stroke requires further rehabilitation with additionally $ 40.000/year [15]. Therefore, a haploidentical HSCT is about equivalent to 3 years transfusion program with chelation. Health economically a significant benefit, not to mention the social rehabilitation and reintegration of these patients. The proof of concept will allow

	physicians to refer SCD patients earlier to transplant, avoiding many of the SCD related complications as well as the transplant-related morbidity and mortality that rises with each year of delay [16].

Objectives of clinical trial

An overview of study objectives and corresponding outcome parameters is shown in the following table:

Study Objectives	**Outcome Parameters**
Primary	
The objective of this phase II trial is to prove that EFS following T-Haplo-SCT (experimental group E) is non-inferior to matched sibling donor (MSD) HSCT (reference group R) as well as evaluation of safety/tolerability and feasibility of haploidentical PBSC grafts depleted of TCR$\alpha\beta$ and CD19 cells in adult and paediatric patients with sickle cell disease: Incidence of disease free survival (DFS), primary graft failure (pGF) and grade III–IV acute graft-versus-host disease (GVHD) until Day 100 post-transplantation and rejection.	**Acute graft-versus-host disease grade III–IV:** defined as GVHD occurring within 100 days after SCT. Severity graded according to MAGIC criteria (*appendix B.1*): - Incidence of aGVHD grades III–IV - Time until occurrence of aGVHD grades III–IV **Primary graft failure (pGF):** ANC <0.5 × 10^9/L by Day 28 and platelets <20 × 10^9/L (Hemoglobin <8 g/dL Is omitted due to inclusion of donors with SCD heterozygosity) and/or disease recurrence **Death (from any reason)** **Moderate and severe chronic graft-versus-host diseases (cGVHD):** Incidence/severity graded according to the NIH Consensus Guidelines fom 2015 [17] (*appendix B.2*)
Secondary	
Safety outcome parameters	
- Incidence of grade I-II acute GVHD until Day 100 post-transplantation	- GVHD grade I, occurring within 100 days after SCT. Severity graded according to MAGIC criteria (*appendix B.1*) • Incidence of GVHD grade I-II • Time until occurrence of GVHD grade
- Incidence and severity of chronic GVHD 1 year after omission of immunosuppression	- Chronic graft-versus-host disease: Incidence/ severity graded according to the NIH Consensus Guidelines fom 2014 [17] (*appendix B.2*)
- Incidence of TRM at all visits throughout the study	- TRM (treatment-related mortality): defined as death between day of transplantation (Day 0) and day of assessment, not due to disease recurrence and considered related to treatment by the investigator
- Transplant-related morbidity and early complications	- Days of (re)hospitalization assessed at Day 28, Day 100 and after 1 and 2 years
- Incidence and severity of acute infusion related toxicities	- Infusion related toxicity: maximum toxicity on the days of transfusion evaluated by measuring vital signs prior to and at different times after

	transfusion
Secondary graft failure from Day of successful engraftment until 12 months post-HSCT	- Secondary graft failure (sGF): ANC <0.5 × 10^9/L after initial engraftment not related infection, or drug toxicity, unresponsive to growth factor therapy and/or other intervention.
- Incidence and severity of transplant-related neurotoxicity and PRES	- Incidence of headaches, seizures, visual disturbance, cortical blindness, aphasia, ataxia and image-proven posterior reversible encephalopathy syndrome (PRES) assessed according to the ASTCT consensus grading for neurologic toxicity (*appendix B.7*).
Feasibility outcome parameters	
- Neutrophil and platelet engraftment from Day 0 to Day 28	- Neutrophil engraftment: cell counts determined by flow cytometry, measurements of ANC ≥500/µL on three consecutive days and - Time to neutrophil engraftment as time from last HSCT to engraftment - Platelet engraftment: cell counts determined by flow cytometry, measurements of platelet count ≥20,000/µL on three consecutive days and without platelet transfusion support for seven days - Time to platelet engraftment as time from last HSCT to engraftment **Recorded day is the first of the 3 consecutive days**
- Overall survival at Day 100 and after 1 year	- Overall survival rate (OS): time from transplantation to death or last follow-up
- Disease-free survival at Day 100 and after 1 year	- Disease-free survival (DFS): minimum time to relapse/ recurrence, to death or to the last follow-up
- Transfusion requirement from Day 0 to Day 100	- Number of thrombocyte transfusions after transplantation - Time to last thrombocyte transfusions from Day 0. - Number of erythrocyte transfusions after transplantation - Time to last erythrocyte transfusions from Day 0. - Number of transfusions of other blood products after transplantation - Time to last transfusions (other blood products) from Day 0.
- Quality of life at baseline, Day 100 and after 1 year	- EQ-5D for adults (age ≥18 years), PedsQL for pediatric patients (age <18 years) and FACT-BMT (age ≥18 years) - At baseline, Day 100 and after 1 and 2 years
Laboratory outcome parameters (additional central assessments)	

- Donor chimerism	Assessed by PCR-analysis of peripheral blood samples. Recommended time intervals for chimerism analyses are: - A weekly total chimerism analysis during inpatient care starting with engraftment - A biweekly total chimerism analysis therafter until weaning of immunosuppression, if chimerism remained stable - Again a weekly total chimerism analysis from start to completion of the weaning process of immunosuppression - A monthly total chimerism analysis thereafter until the end of trial
- Reconstitution of T, B, NK and T regulatory (Treg) cell subsets by immune cell phenotyping	- Immune cell phenotyping of T, B, NK and Treg cell subsets according to institutional guidelines for assessment of immune reconstitution (Recommended: Cell counts of $CD3^+$, $CD4^+$, $CD8^+$, $CD3^+CD56^+$, $CD3^+TCR\alpha\beta$, $CD3^+TCR\gamma$ T cells, naive $CD4^+TCR\alpha\beta$, memory $CD4^+TCR\alpha/\beta^+$, naive $CD8^+TCR\alpha\beta$, memory $CD8^+TCR\alpha\beta$, DN $TCR\alpha\beta$, B-cells, NK cells - Samples collected: • before start of conditioning • monthly starting with engraftment until 1 year post-HSCT
Safety parameters: adverse events, concomitant medication	
- Infections: Incidence of CMV, ADV, EBV and aspergillus, as well as other viral, bacterial and fungal infections	- Recurrence or newly occurring infectious diseases: CMV, ADV, EBV, HHV6, BK virus aspergillus and other at weekly intervals until 1 year post-transplantation. - Number of viral reactivations of CMV, ADV, EBV, HHV6, BK virus - Fungal diagnostic when expected clinically, followed weekly until complete resolution.
- Incidence, severity and type of adverse events/serious adverse events	- Documentation of serious adverse events throughout the study - Documentation of 'treatment-related toxicity, known or unknown' from Day –12 to Day 0 (during conditioning and prior to stem cell transplantation) - Documentation of adverse events from Day 0 until 24 months post-HSCT
- Vital signs and physical examination	- Vital signs and physical examination including Karnofsky/Lansky index throughout the study
- Safety laboratory (blood count, blood chemistry)	- Laboratory values for clinical chemistry and complete blood counts at baseline and from Day 4 to Day 100
- Concomitant medication	- Documentation of concomitant medication

	from baseline until Day 100 - Documentation of new treatment with cellular products (erythrocytes, thrombocytes or virus-specific T cells, VST) after Day 100
- Fertility	- Assessment of fertility via imaging and laboratory parameters before, at 1 and 2 years post-HSCT according to the institutional recommendations or following the trial specific recommendations
Primary endpoint	**Primary efficacy endpoint:** Composite Endpoint: Event free survival (EFS) measured from HSCT until event. Event is defined as aGVHD (Grade III - IV), cGVHD (moderate/severe), primary graft failure (pGF), or death (from any reason).
Secondary endpoint	**Key secondary endpoint(s):** (i) Overall survival (OS); (ii) secondary Graft failure (sGF); (iii) Immune reconstitution; (iv) Quality of life (QOL); (v) Fertility
Inclusion criteria	• Age 1yr to 35yrs • Homozygous hemoglobin S disease or heterozygous hemoglobin SC or S 0/+ • Study specific consent given • Preexisting **severe** or **moderate** SCD related complications: o Clinically significant neurological event (stroke) or deficit o Silent crisis, neurocognitive deficit o Pathological angio-MRI with TOF Sequence o TCD velocity >200 cm/s at 2 occasions >1 month apart o More than **5** vaso-occlusive crises (VOC) in the past 1 year **or** more than **20** VOC in a lifetime o Two or more episodes of acute chest syndrome (ACS) in a lifetime or one episode of ACS in the past 24 months o Chronic transfusion requirement **or** more than 8 transfusions **or** one exchange transfusion in a lifetime o Transfusion-refractory allo-immunization o More than five SCD-related hospitalizations in a lifetime o Beginning pulmonary hypertension o Osteonecrosis at more than 2 sites o Beginning SCD Nephropathy o Recurrent priapism (>2)

Exclusion criteria

- Karnofsky or Lansky Performance Score < 70%
- Patients with donor-specific antibodies (DSA) against the potential stem cell donor by either
  - Cell-based crossmatched assays (Complement-dependent cytotoxicity; CDC) or
  - Flow cytometry crossmatch test or
  - Solid-phase immunoassays (SPI) or
  - Modified SPI such as C4d and C1q assays

  Whichever method the participating center is experienced in.
- Patients with **a haploidentical donor** with major AB0 incompatibility defined according to EBMT Handbook, Edition 2019 Tab 23.1.:

ABO incompatibility	Recipient	Donor
	0	A
	0	B
Major	0	AB
	A	AB
	B	AB

- **Cardiac function:**
  - Ejection fraction at rest <45.0% on echocardiography or
  - Shortening fraction of ≥ 27.0% by echocardiogram or radionuclide scan (MUGA)
  - Patients with > grade II hypertension by Common Toxicity Criteria (CTC)
- **Renal function:**
  - Estimated creatinine clearance (for patients > 12 years) lower than 50.0 mL/minute
  - for pediatric patients (> 1 year to 12 years), GFR estimated by the updated Schwartz formula < 90.0 mL/min/1.73 m2. If < 90 mL/min/1.73 m^2, renal function must be measured by 24-hour creatinine clearance or nuclear GFR and must be > 70.0 mL/min/1.73 m^2 for inclusion or
  - Creatinine clearance below threshold defined for stem cell transplantation according to local clinical standard
- **Pulmonary function:**
  - DLCO >50% (adjusted for hemoglobin), and FVC and FEV1≥50%; children unable to perform for PFTs, O2 saturation <92% on room air.
- **Liver function:**
  - Direct bilirubin > 3 x the upper limit of normal (ULN) (unless elevated bilirubin is attributed to Gilbert's Syndrome) and ALT/AST > 2.5x the ULN.
  - Chronic active viral hepatitis
- Women who are pregnant (positive serum or urine βHCG) or breastfeeding.

  Note: Women of childbearing potential must have a negative serum pregnancy test at study entry.
- Adults of reproductive potential not willing to use an effective method of birth control during study treatment up to the end of follow-up >24 months after HSCT)
- History of uncontrolled autoimmune disease or on active treatment
- Patient unable to comply with the treatment protocol
- Prior autologous or allogeneic HSCT
- Vaccination with a live virus vaccine during the trial

	• HIV infection • Patients with a history of psychiatric illness or a condition which could interfere with their ability to understand the requirements of the study (this includes alcoholism/drug addiction) • Patients unwilling or unable to comply with the protocol or unable to give informed consent. • Concurrent severe or uncontrolled medical disease (e.g. uncontrolled diabetes, congestive heart failure, myocardial infarction within 6 months prior to the study, unstable and uncontrolled hypertension, chronic renal disease, or active uncontrolled infection) which by assessment of the treating physician could compromise participation in the study. • Patients with prior malignancies, except resected non-melanoma or treated cervical carcinoma in situ. Cancer treated with curative intent >5 years previously will be allowed. Cancer treated with curative intent < 5 years previously will not be allowed unless approved by the Protocol Officer or one of the Protocol Chairs.
Evaluation of efficacy	Disease-free and GVHD-free survival 1 year after omission of immunosuppression
Evaluation of safety	**Assessment of Safety:** Transplant-related morbidity and mortality (TRM), graft failure (GF), incidence of clinically relevant viral reactivation, adverse effects (AE), incidence of early and late complications. Composite endpoints that not only encompass mortality and relapse, but other critical post-transplant events such as GVHD, are pivotal for outcome measures in non-malignant diseases such as SCD to quantify survival without significant morbidity after HSCT [18]. Emphasis is placed on fertility preservation as being a major criterion for patients with non-malignant diseases. The remaining endpoints are standard endpoints for HSCT trials.
Investigational medicinal product	TCR$\alpha\beta$ and CD19 depleted haploidentical stem cell transplantation in patients with sickle cell disease
Treatment / Procedures	Patients who fulfill inclusion criteria will be stratified according to donor availability. Patients with a matched sibling donor (MSD; defined as 8/8 (or preferably 10/10 allelic match) will be stratified into the reference arm. All others into the experimental arm R. All patients will undergo a myeloablative conditioning regimen prior to the intravenous infusion of the graft. The conditioning regimen for both arms is identical with the exception that anti-thymoglobulin (ATG-Neovii®) is dosed at 15mg/kg for T-haplo and 10mg/kg for MSD HSCT and given upfront on day -10 to -8 in T-haplo and on day -3 to -1 in MSD HSCT. Chemotherapy consists of thiotepa 2 x 5 mg/kg, fludarabine 4 x 40 mg/m^2 and treosulfan 3 x 14 g/m^2, given between days -10 and -2. This conditioning is well established and safe, myeloablative and highly immunosuppressive. In contrast to busulfan, treosulfan is well tolerated, does not pass the blood-brain barrier and has a lower incidence of endothelial complications such as sinusoidal obstruction syndrome (SOS/VOD). Graft content and engineering: a/ß depleted T-Haplo-SCT: CD34 >1x10^7/kg, CD3 <5x10^4/kg; CD20 ≤1×10^5/kg. BM: CD34 >2-8x10^6/kg. The duration of treatment consists of app. 10 days conditioning and stem cell infusion, followed by an in-patient follow-up of app. 20-40 days until the patient is transfusion independent and can tolerate oral intake and medication. Total follow-up for this trial is approximately 2 years which permits to assess all trial relevant parameters. Patients will then enter the institutional long-term follow-

	up program of their respective institutions according to institutional guidelines and legal requirements. Donors: Since stem cell transplantation is the only potentially curative therapeutic option for the critically ill patients in this study it is planned independently of this trial. All procedures related to donors (stem cell mobilization and stem cell apheresis) will therefore be performed according to clinical routine and independent of this study. The only procedure for donors required to allow the patients' study enrolment is the collection of a reference blood sample. Accordingly, the donor has to consent to data transfer and collection and genetic analysis of one blood sample. For information about these requirements and collection of the blood sample the donor has to visit a study center once. Preparation of IMP: Stem cell apheresis of the donor will be depleted of TCRαβ and CD19 positive cells using the Miltenyi CliniMACS® or the Prodigy® TCR$\alpha\beta$/CD19 systems. Stem cell apheresis and subsequent depletion will continue until a post selection target of CD34 >1x10^7, CD3αβ <5x10^4, CD20 ≤1×10^5 per kg BW of the recipient is reached following at least one but not more than three stem cell apheresis procedures. No upper limit of CD34+CD45+ progenitor cells has been defined. It is not permitted to exceed the target cell number of 5x10^4 TCRαβ T cells/kg BW. For MSD: Bone marrow is collected according to standard institutional procedures with a yield for CD34 > 2-8x10^6 per kg BW of the recipient. **Fertility** With the advancement of HSCT to the point of very low transplant-related mortalities, the question of fertility preservation is increasingly important, in particular for patients with non-malignant diseases. Therefore, we will explore prospectively all possible pre-transplant procedures for fertility preservation and use standardized protocols for assessment of puberty and fertility post-HSCT. **Pharmacokinetics and pharmacodynamics of Grafalon** Aim is to establish an evidence-based dosing regimen of Grafalon® (ATG Neovii) using pharmacokinetic and pharmacodynamic studies to understand inter-individual variability to optimize dosing and reach an improved clinical outcome in patients treated with HSCT. Accompanying Projects: **Chimerism and split-chimerism** Chimerism and split-chimerism analyses are of utmost importance since substantial consequences such as rejection and disease reoccurrence can be predicted. Classically, a mixed chimerism is treated with withdrawal of immunosuppression and donor lymphocyte infusions (DLI) to achieve full chimerism. According to our experience, in T-Haplo HSCT of non-malignant diseases both treatment options can be wrong and harmful. In T-depleted HSCT, immaturity of donor T-cells requires a paradoxical extension of immunosuppression rather than withdrawal, in addition DLI can induce GVHD. Also, in HSCT of SCD, erythroid precursors have an engraftment advantage so that these patients can present with a 'dissociated' split chimerism with a low percentage of donor T cells and a full donor erythroid engraftment. This situation does not need any intervention. Split-chimerism analyses can therefore reveal the proper cellular constellation of donor and recipient. Tests are done classically via PCR-based STR analysis, but this technique is labor-intensive and expensive. We will further develop and standardize the FACS-based split chimerism analysis using a MACSQuant® flow cytometer on haplotype HLA antigens. The goal is to be able to offer this technique to the majority if not all haploidentically transplanted patients in this trial using newly

	available and secondarily labelled anti-HLA antibodies. Next to costs and labor, FACS based chimerism analysis will allow a faster, cheaper, widely available and a detailed analysis of hematopoietic subsets during reconstitution. During the trial, samples from patients expressing a mixed chimerism will be analyzed centrally in parallel via FACS and PCR, in order to establish validity of the FACS-based system.
Statistical methods	Efficacy/test accuracy: The objective of this phase II trial is to prove that EFS following T-Haplo-SCT (experimental group E) is non-inferior to matched sibling donor (MSD) HSCT (reference group R). Typically, phase II-trials are single-arm trials testing an experimental treatment against a historic reference. However, 'historic' data on MSD HSCT are not uniform enough to be used as unbiased reference for T-Haplo-SCT in SCD. Thus, group R will be included as prospective reference group in a two-arm design. Experimental group E (reference group R) is defined as patients with no MSD (with MSD) who will be treated with T-Haplo-SCT. Reference group R and experimental group E will thus be transplanted almost identically. In particular, treatment arm allocation is done according to availability of MSD. A design based on randomized allocation of treatment arm within the group of patients with available MSD was discarded for ethical and economic reasons, since donor availability is rare (<20%) and since the latter would imply withholding MSD HSCT for patients with available donors. The null hypothesis of the primary endpoint is: EFS of SCD patients treated with the experimental intervention is non-relevantly inferior to EFS in the prospective reference group. In formulas, $H_0: \omega \geq \omega_0$, where ω is the true hazard ratio (experimental vs. reference group). The non-inferiority margin is $\omega_0 = 1.13$ (hazard ratio: experimental vs. reference group). A one-sided significance level α=0.05 and Power of 80% are stipulated. An adaptive design with one interim analysis will be performed with the possibility to recalculate sample size based on the observed interim data. The characteristics of the adaptive design are determined according to the inverse normal method. The bounds of the 2-step adaptive design result from a group-sequential design without futility stop according to Wang and Tsiatis[19] with shape parameter $\Delta = 0.23$ and information rates 0.33 and 1 of the analyses. The interim and final analysis are done at 9 and 27 accumulated events pooled over both groups, respectively. Assumptions on the 2-year EFS-rates: 73% for prospective reference group 90% for experimental group Expected allocation ratio: 4:1 (patients without vs. with MSD) Accrual period: 4 years Assumed follow-up period: 2 years Drop-outs: 2-year loss to follow-up rate: 5% Description of the primary efficacy/test accuracy analysis and population: The null hypothesis of the primary endpoint will be analyzed by one-sided Wald test in a stratified Cox-regression with treatment group (experimental or reference) as binary covariate, stratified by age group (<16 or ≥16 years at transplantation). The primary analysis will be based on the intention-to-treat (ITT) analysis set. A sensitivity analysis will be conducted on a per-protocol analysis set. Additionally, a confirmatory confidence interval for the true hazard ratio is constructed Safety: A DSMB report will be provided annually. As essential part of each DSMB report, the rate of transplant-related mortality (TRM) and acute graft-versus-host disease (aGVHD) is intended to be compared between both arms by one-sided tests of rates using a group-sequential Pocock type α-spending approach. If any of the analyses shows a relevant inferiority of the experimental

	treatment, the result will be judged as a critical safety finding to be discussed with the DSMB. Secondary endpoints: Analyses are supplementary exploratory and are performed by: (i)-(ii) log-rank test, (iii)-(iv) test of rates, (v) Mann-Whitney U test, (vi) descriptive
Safety monitoring and statistical stopping guidelines	Patient safety during the study will be assessed continuously throughout the study by monitoring incidence and severity of aGVHD and incidence of TRM until Day 100 post-transplantation and type of AE. In addition, changes in findings of physical examination, vital signs and clinical laboratory results (complete blood count, differential and platelet count and blood chemistry) will be evaluated for the times defined as appropriate. Each case of aGVHD grade III–IV and each case of TRM have to be reported immediately and will be announced to the DSMB. Pre-defined statistical stopping guidelines for pGF and TRM have been implemented and will be used as trigger for consultation with the DSMB to decide about further study continuation. In case of premature study termination patients included will be observed until their individual end of treatment as scheduled. aGVHD grade II-IV will also be analyzed and assessed by the DSMB for decision on the conditioning regimen and post-HSCT immune therapy.

2. INTRODUCTION

2.1. Sickle cell disease

Sickle cell disease (SCD) is an autosomal recessive, progressively debilitating multi-organ disease, resulting in a significantly shortened life expectancy. SCD represents a major public health concern, recently recognized by the World Health Organization (WHO) and the United Nations (UN) as a global health problem. At least 2% of the world population carry a hemoglobin S variant causing over 80% of hemoglobin disorders which contribute to the equivalent of 3.4% of mortality in children aged under 5 years worldwide. Annually there are over 300.000 affected conceptions or births [20]. The exact number of patients treated in many western countries is unknown, but awareness is growing and patients registered at pediatric hematology centres are rising exponentially [1]. In children, stroke is the major complication of SCD and SCD is the most frequent cause of stroke in children. Thirty percent develop silent strokes leading to cognitive impairment in children and an impaired quality of life [2, 21].

SCD is also a major socioeconomical problem, even in developed countries. From 1989 through 1993, an average of 75,000 hospitalizations due to SCD occurred in the United States, with approximately $475 million (http://www.cdc.gov/ncbddd/sicklecell/data.html) of health care related expenses. The estimated lifetime cost of medical care for SCD patients was 460.151$ per SCD patient [14], chronic transfusions and iron chelation excluded which adds approximatey 40.000$/year. A stroke requires further rehabilitation costs of $ 40.000/year [15].

The mainstay of treatment is erythrocyte transfusions and symptomatic pain management. More than 90% of adults receive at least one transfusion in their lifetime. Indications for chronic transfusion most frequently relate to stroke prevention, given that patients with previous strokes have a high risk for recurrence[4]. However, discontinuation of prophylaxis is associated with stroke recurrence and increased mortality [5]. Even despite hyper-transfusion the disease progresses with 17% recurrent strokes and 31% new silent infarcts [9].

Hydroxycarbamide (HU) was shown to induce hemoglobin F (HbF) in SCD and reduced the median incidence of painful crisis per year by 44% [7]. In children with known cerebrovascular disease, a benefit of HU for primary and secondary stroke prevention was assumed, but HU and transfusions have to be continued life-long with the downside of iron overload and the long-term adverse effects of chemotherapy, in particular in children. HU is not always tolerated well and the compliance for a life-long medication is poor, in particular in adolescents and young adults in childbearing age with regard to fertility and family planning. Inasmuch HU starting in infancy has a positive impact on the systemic vascular complications remains to be demonstrated (see below).

SCD is a two-faced disease. In low and middle income countries (LMIC) SCD presents as the classical text book disease with an exceedingly high infant mortality mostly due to uncontrolled infections and vaso-occlusive crises. In industrialized countries infant mortality is almost abolished due to conventional measures such as patient and parent education, immunization, aggressive anti-infectious therapy, adequate pain management and HU [2].

Despite significant improvements in preventive and therapeutic modalities in the industrialized countries, the morbidity and mortality of this chronic multi-organ disease has not improved significantly in the last decades [21 22] with an average life expectancy between 40 to 50 years and a 30% to 50% disability and unemployment rate. The median survival for adults with SCD persists to remain on average 20 years shorter than for African Americans living in the United States [10].

Therefore, despite these highly effective conventional measures, the disease seems to progress unopposed and the classical presentation is replaced by a systemic vasculopathy leading to acute and chronic cardio-pulmonary failure, renal failure and thromboembolic events as the major cause for the limited overall survival and the progressively morbidity compared to an age matched population.

2.2. Hematopoietic stem cell transplantation (HSCT)

Hematopoietic stem cell transplantation (HSCT) is a treatment option for a growing number of diverse

congenital and acquired disorders, both malignant and non-malignant. The activity survey of the European Group for Blood and Marrow Transplantation (EBMT) reports the performance of 45,223 HSCTs in 2017 in Europe [23]. Conditions treated comprise hematological malignancies, solid tumors and others. Overall 635 patients with hemoglobinopathies were transplanted in 2017 (1,4%) with 215 patients with sickle cell disease (SCD). Generally, HSCT is still associated with serious risks for the patients and a high incidence of complications. It remains therefore restricted to patients with life-threatening diseases. However, in a number of hematological indications HSCT alone offers a curative option for several non-malignant conditions such as congenital or acquired stem cell defects and immunodeficiencies (e.g. SCID and congenital neutropenia), hematologic diseases (e.g. paroxysmal nocturnal hemoglobinuria, aplastic anemia, and hemophagocytic lymphohistiocytosis) and hemoglobinopathies. Rationales for the use of HSCT in the non-malignant indications are mostly the restoration of a functional immune or hematopoietic system and the supplementation of defective enzyme activities by the transplanted blood cells.
To avoid serious graft-versus-host disease (GVHD) and graft failure (GF) in all allogeneic stem cell transplantation settings it has long been inevitable to find donors with the highest possible match to the respective recipients with respect to essential cellular markers, especially HLA-characteristics. The therapeutic use of HSCT thus is limited by the availability of a suitable HLA-matched donor. A matched related donor can be found for only 30% of the patients, 70% of the patients thus have to rely on finding a matched unrelated donor. Though donors can be identified for most of these patients, the search takes at least several weeks if not months. However, in patients with SCD donor availability is limited by a MSD or a matched unrelated donor (MUD) availability of less than 20% [11, 24-26].
As an alternative, haploidentical HSCT using closely related, but only partially matched family donors have been exploited in several therapeutic settings, recently. In theory, virtually every patient has a potentially suitable haploidentical related donor—parent, sibling or child—and thus a successful strategy for haploidentical allogeneic hematopoietic cell transplantation (HHCT) may clearly be the solution for the 'lacking donor' problem. Yet, once again in only partially matched donors and recipients the difficulties of resulting GVHD and GF arise, and initially trials of HHCT were complicated by a high incidence of GVHD, engraftment failure, and infectious complications resulting in an unacceptably high treatment related morbidity and mortality [27] efforts have therefore been directed on developing therapeutic strategies to minimize these complications. Graft rejection and GVHD are primarily mediated by T cells of host and donor. Therefore, attempts to overcome the HLA-barrier have focused on strategies for effective host and graft T cell depletion.

CD3+ and CD19+ Depleted Stem Cell Grafts

The aim of another therapeutic approach therefore was to develop a strategy to improve engraftment independent from the infused stem cell doses. First promising experiences were published for a paediatric population [28].
Two phase I/II trials were initiated (adult patients with high risk disease of AML, ALL, NHL, MM and CML and paediatric patients suffering from acute lymphatic and myeloid leukemias, MDS, solid tumors and non-malignant diseases). No G-CSF was administered post-transplantation and mycophenolate mofetil (MMF, 15 mg/kg bid) was used only if the T-cell content in the graft exceeded 5×10^4 CD3+ cells/kg. The regimen was well tolerated, and engraftment was rapid (median time to >500 granulocytes/µL 13 days and to >20,000 platelets/µL 11 days). Furthermore, all but one patient engrafted with full donor chimerism by day 14 to 28 post-transplantation. In the trial with paediatric patients graft rejection occurred in 13%. After total nodal irradiation (TNI) based reconditioning and second haploidentical stem cell donation, final engraftment in paediatric patients was achieved in 100%. However, in adult patients, immune reconstitution was delayed due to the profoundly T cell depleted grafts. NK cell reconstitution was fast, probably due to the high NK-cell content of the CD3+/CD19+ depleted grafts.
In our hands the CD3+ CD19+ depleted stem cell grafts were used for haploidentical HSCT in 15 patients with sickle cell disease with a median age of 21 years. The median follow-up was 26 (9–62) weeks with an OS, DFS and TRM of 13 (87%), 13 (87%) and 2 (13%), respectively. No patient rejected the graft. The main AE were bone pain during engraftment in 10 patients and viral reactivation in 9. No grade III-IV aGVHD and cGVHD was mild to moderate, observed predominantly in patients >18 years and resolved mostly within 18 months post-HSCT [29].

In conclusion, the applied regimen allowed haploidentical HSCT in an older or heavily pretreated patient population with SCD. However, several factors still needed to be improved: the reconstitution of T cells,

especially the recovery of CD4 cells was poor, and the rate of viral reactivation remains critical.

TCRαβ and CD19 Depleted Stem Cell Grafts

The significant impact of graft composition and conditioning regimen on engraftment has already been demonstrated by the fast engraftment kinetics observed in patients receiving CD3/CD19 depleted grafts compared to merely CD34 enriched grafts. Mainly the subsets of CD3 cells with TCRαβ receptors mediate graft-versus-host activity while, contrarily, CD3 cells with TCRγδ receptors show the highly interesting graft-versus-tumor activity [30-33].

Furthermore, recent studies have revealed the existence of CD34 negative hematopoietic stem cells (HSC), which are probably precursors of CD34+ HSCs with a high repopulating capacity [34]. Additional graft facilitating cells have also been defined, such as CD8- positive T-cells, monocytes and antigen-presenting cells (APCs) [34-39]. The immunoregulatory properties of CD34+ HSC have been demonstrated previously [40, 41]. An efficient depletion of GVHD-mediating T cells presenting CD3 and TCRαβ as surface markers is imperative to prevent aGvHD. On the other hand, the remaining transfusional product has to have a high number of CD34+ HSC. Further improvement of the cell product is achieved by the depletion of CD19 B cells because this reduces the risk for post-transplantation EBV-related lymphoproliferative disorder (PTLD). PTLD has previously been a major risk in transplantation settings [42]. In consequence, a new cell sorting strategy for processing of the cell grafts has been developed using again the Miltenyi CliniMACS® cell sorting system. The HSC grafts are selectively depleted of TCRαβ and CD19 cells by using paramagnetic microbeads in a single processing step and the resulting cell grafts are rich in a variety of blood cells with diverse immunological properties [43].

In all patients transplanted with TCRαβ and CD19 depleted grafts, engraftment was rapid. Immune recovery was markedly improved compared to patients with CD3/CD19 depletion and at day 100 TCRαβ cells were predominant, nevertheless. Furthermore, V beta spectratyping showed a broad spectrum of T-cell receptors early after transplantation.

In our series of 8 consecutively transplanted SCD patients according to an identical transplant regimen as we used with the CD3+/CD19+ depleted grafts the OS, DFS, TRM and the incidence of acute and chronic GVHD was identical. No rejection occurred in these advanced stage SCD patients [29].

In summary, in all pilot patients treated so far, rapid and sustained engraftment, rapid immune reconstitution, and a low incidence of GVHD were observed. Cell sorting using the CliniMACS® device proved to be efficient with a high TCRαβ log depletion while good recovery rates for HSC and innate effector cells were observed with a high viability of the resulting cells in the transplant. Therefore, these results are very encouraging for HSCT of advanced stage SCD patients.

2.3. Current treatment approaches for patients with sickle cell disease

2.3.1. Conventional therapy and supportive care

Hydroxycarbamide (HU) was shown to induce Hemoglobin (HbF) in SCD [6] and reduced the median incidence of painful crisis per year by 44% [44]. In children with known cerebrovascular disease, a benefit of HU for primary and secondary stroke prevention was assumed [8], but HU and transfusions have to be continued life-long with the downside of iron overload and the long-term adverse effects of chemotherapy, in particular in children. More than 90% of adults receive at least one transfusion in their lifetime. Indications for chronic transfusion most frequently relate to stroke prevention, given that patients with previous strokes have a high risk for recurrence [4]. However, discontinuation of prophylaxis is associated with stroke recurrence and increased mortality [5]. Despite hyper-transfusion the disease progresses with 17% recurrent strokes and 31% new silent infarcts [9]. More recently several novel SCD therapies have been developed to target specific pathophysiological mechanism intended to either prevent or abort approaches to vaso-occlusion. L-glutamine has been shown to increase the proportion of the reduced form of nicotinamide adenine dinucleotides in sickle cell erythrocytes and was the first substance licensed by the FDA in SCD in the last 20 years [45]. In late-phase development for vaso-occlusion are prevention include voxelotor (GBT440), which elevates hemoglobin oxygenation, and prasugrel, a platelet activation inhibitor. Other drugs in part already licensed are crizanlizumab, an anti-P-selectin monoclonal antibody and rivipansel (GMI-1070), a pan-selectin inhibitor, which appear to reduce the frequency of vaso-occlusion, hospitalization time and opioid use. Biological molecules might increasingly play a primary role in SCD therapy, either for prevention or in acute crises and either in

combination with HU or to replace it not only in patients with serious adverse reactions, females of child bearing age and wish for pregnancy et al, but in general due to a higher efficacy, lower adverse event rate and therefore better compliance [46, 47]. Inasmuch these new and probably expensive medications are capable of preventing the longterm chronic vasculopathy, will be seen in the future.
The major benefit of these novel treatment options could be the optimal preparation of patients as a 'bridge' for transplant in order to reduce transplant related morbidity.

2.3.2 Hematopoietic stem cell transplantation

HSCT is currently the only curative option for SCD but less than 20% of SCD patients have a MD donor available [11]. So far, all curative approaches beyond MSD HSCT at young age are non-satisfactory. Beyond 15 yrs. OS, DFS and the incidence of GVHD is significantly worse (see below) [48]. With the lack of a suitable donor for the vast majority of patients, the major question of this trial is, if a α/ß T-depleted HSCT can be a valid alternative to MSD HSCT. The main challenge in non-malignant diseases is, to offer a safe and GVHD-free HSCT without rejection. This is a particular challenge in SCD patients suffering from systemic vasculopathy, a high risk of alloimmunization and rejection due to multiple transfusions. Surveys among SCD patients revealed that GVHD was an unacceptable complication (80%) and should be avoided at all cost [12, 13]. The main questions therefore are: Safety of an α/ß T-depleted haploidentical HSCT; Incidence of acute and chronic GVHD; Rate of rejection; Immune reconstitution; Fertility and quality of life post-HSCT.

The cost for a haploidentical HSCT is about equivalent to 3 years of a transfusion program with chelation. Health economically a significant benefit, on top of the social rehabilitation and reintegration of SCD patients. The proof of concept will allow physicians to refer SCD patients earlier to transplant, avoiding many of the SCD related complications as well as the transplant-related morbidity and mortality that rises with each year of delay [49].

2.3.2.1 Matched sibling donor

To date, allogeneic hematopoietic stem cell transplantation (HSCT) from an HLA-identical sibling donor (MSD) is the only curative option offered currently only to patients with severe SCD. The reported overall survival (OS) and event free survival (EFS) exceeds 90% and 80%, respectively, and EFS greatly improved to 95% for patients transplanted after 2006 [16, 50].
However, this standard approach is limited by a MSD availability of less than 20% [11, 24-26]. Also, the excellent outcomes reported for MSD HSCT are only reproducible in infants and children below 5 years of age. With aging patients beyond adolescents (>15years) the reported outcomes are declining to an OS, DFS and an incidence of cGVHD of 88%, 81% and 20% [48].

2.3.2.2. Matched unrelated donor

Due to the lack of a MSD, HSCT using alternative donors (MUD, MMUD, haploidentical and CB) are seriously considered and explored. While the probability to find an unrelated 8/8 HLA donor and 6/6 umbilical cord blood (UCB) donor is around 16% and 2% to 6% respectively, the likelihood increases to 76% and 24% to 58% when considering 7/8 or 5/6 UCB products, respectively [26]. MSD and MUD donors are generally considered equivalent in OS and DFS in patients transplanted for ALL[51, 52], but the current results in SCD are not quite as satisfactory, in particular with regard to acute and chronic GVHD (aGVHD, cGVHD). The American cooperative group recently completed the only matched unrelated donor (MUD) transplantation trial for SCD [53] where a high rate of acute and severe cGVHD (62% at one year) was observed, responsible for 7 out of the 8 deaths. A registry-based analysis by Eapen et al reported in 111 patients an OS, DFS and an incidence of cGVHD of 82%, 69% and 31% [54].

2.3.2.3. Haploidentical HSCT

Haploidentical donors (HID) are mostly available for the majority of patients. 44 Partially HLA-mismatched first-degree related donors increase the donor pool to an average of 2.7 donors per patient [55]. In addition to immediate donor availability, the incidence of aGVHD and cGVHD seems to compare favorably with MSD or MUD donors [29, 56-59]. Haploidentical related donor HSCT is currently being

conducted in experienced centres. Currently two haplo-HSCT concepts are used worldwide: T-cell depleted peripheral blood HSCT (CD3/CD19 or TCRαβ/CD19; T-haplo-HSCT) and post-transplantation cyclophosphamide (post-CY-haplo-HSCT).

2.3.2.3.1. Haploidentical with post-transplant cyclophosphamide

Post-CY-haplo-HSCT seems very intriguing due to its simplicity, ubiquitous availability, and fast immune recovery after the infusion of a non-manipulated graft. Activated T-cells are depleted due to a lack of aldehyde dehydrogenase and sensitized for cyclophosphamide [60]. The landmark trial from Baltimore enrolled 14 patients, 6 (43%) experienced graft rejection with autologous reconstitution (7, adding a patient with a 5% donor chimerism), but no patient experienced any GVHD and all patients survived [61]. De la Fuente et al published a series of 18 patients. The initial conditioning regimen included ATG, fludarabine, cyclophosphamide, and low-dose total body irradiation. Primary graft rejection occurred in 67% (2/3) triggering the study-stopping rule. To reduce the risk of graft rejection, thiotepa was added to the conditioning regimen. Fifteen patients including two with prior graft rejection, underwent haplo-BMT with a thiotepa-augmented conditioning regimen. After a median follow-up of 13.3 months (IQR 3.8-23.1), 93% (14/15) had >95% stable donor engraftment at 6 months, with 100% overall survival. Two participants had grade III-IV aGvHD, one participant had mild cGVHD, and 86% (6/7) of participants were off immunosuppression therapy by 1-year post-transplant [58]. HSCT performed between 2013 and 2017 within the same transatlantic SCD consortium, 4 patients died of a macrophage activation syndrome (MAS) when in addition to thiotepa a preconditioning with hydroxyurea, hypertransfusion and azathioprine was applied [62]. The registry-based analysis by Eapen et al on post-CY-haplo-HSCT reported in 137 patients an OS, DFS and an incidence of cGVHD of 64%, 49% and 27% [54].

2.3.2.3.2. Haploidentical HSCT with CD3/CD19 or aß/CD19 T cell depleted grafts

So far, all curative approaches beyond a MSD HSCT are not satisfactory. With the lack of a suitable donor for the vast majority of patients, the major question of this trial is, if an α/ß T-depleted HSCT can be a valid alternative to a MSD HSCT. The main challenge in non-malignant diseases is, to offer a safe and GVHD-free HSCT without rejection. This is a particular challenge in SCD patients suffering from systemic vasculopathy, a high risk of alloimmunization and rejection due to multiple transfusions. Surveys among SCD patients revealed that GVHD was an unacceptable complication (80%) and should be avoided at all cost [12, 13].
In a compassionate us pilot trial with 25 patients with advanced stage SCD were transplanted with a CD3/CD19 and TCRαβ/CD19 depleted haploidentical graft in comparison to 13 patients transplanted from a MSD using an almost identical regimen[29]. The conditioning regimen consisted of ATG, thiotepa, fludarabine, and treosulfan. The median follow-up in the T-haplo-HSCT and the MSD patients was 19 (9–62) and 22 (7–60) months, respectively. The OS in the T-haplo-HSCT and MSD was 88% and 100%, respectively. In the T-haplo-HSCT group, two patients succumbed to a CMV pneumonitis and a macrophage activation syndrome (MAS). One patient in the T-haplo-HSCT group requires renal replacement therapy because of BK virus nephritis. None developed grade III–IV aGVHD. In the T-haplo-HSCT and in the MSD, 16% and 15%, respectively, developed a mild or moderate cGVHD. The incidence of cGVHD was age dependent with 33% in patients >18 years versus 4% in younger patients. These results demonstrate the feasibility, safety, and efficacy of T-haplo-HSCT also for adult patients with advanced stage SCD.

2.4. Study rationale

Study rational is to evaluate if α/ß depleted T-Haplo-HSCT can be considered equivalent to a MSD with regard to disease free survival, AE and safety, in order to offer cure for the majority of patients with SCD.

The main questions of this trial are:

- Safety of a α/ß T-depleted haploidentical HSCT
- Incidence of acute and chronic GVHD
- Rate of rejection
- Immune reconstitution

- Fertility

We expect that the use of TCRαβ and CD19 depleted haploidentical cell grafts in combination with the less aggressive and well tolerated conditioning regimen needed for patient preparation will be associated with a low risk of grade III-IV aGVHD and no extensive cGVHD, no GF and increase speed, spectrum and functionality of immune system reconstitution. This is supposed to reduce the incidence of severe infections leading to lower rates of transplantation related mortality (TRM). Preliminary data have shown that the incidence of aGVHD III–IV after transplantation of TCRαβ/CD19 depleted haploidentical grafts was comparable to that seen after transplantation of CD3/CD19 depleted and was not observed in our pilot series of advanced stage SCD patients [29].
The success of this trial could install T-depleted haploidentical HSCT as a curative gold standard for SCD in patients with no MSD availability, in particular when a shorter time to transplant, an accelerated rate of engraftment and a lower rate of acute and cGVHD proofs to approach or excel a MSD donor HSCT with regard to pre-HSCT disease severity.
The benefit of this trial for the individual, suffering from this devastating disease, is the proof that a safe procedure can cure without delay and with a low morbidity and mortality. A successful trial will allow physicians to refer SCD patients earlier to transplant, avoiding many of the irreversible SCD related complications as well as the transplant-related morbidity and mortality that rises with each year of delay [16].
The main study objectives are to demonstrate safety and feasibility of the proposed treatment regimen in a mixed population of adult and pediatric patients with SCD. In order to increase the accessible patient population and to avoid center-specific effects the trial will be conducted in a multicentre design.

2.5. Risk-benefit-assessment

Stem cell mobilization and apheresis are not part of the study-specific procedures. Donors will be informed separately and according to institutional guidelines of the respective collection center regarding potential risks and side effects. The only study-specific procedure for donors is the additional collection of blood samples which is not associated with any serious risk.

2.5.1. Potential study specific benefits for recipients

The transplantation of TCRαβ and CD19 depleted haploidentical stem cell graft offers potential cure from SCD to participants with no available MSD. Patients with a MSD benefit from a prospective, controlled and safe transplant procedure.

2.5.1.1. Low GVHD rates

TCRαβ cell depletion is expected to result in similarly low GVHD rates compared to CD34 positive selection and CD3/CD19 depletion and thereby also enabling accelerated immune reconstituion.

2.5.1.2. Expedited immune reconstitution

The selective removal of TCRαβ cells enables a stem cell graft that approximates a regular T cell replete graft as much as possible, retaining potentially beneficial effector cells like CD3+ positive NK-cells and CD3+ positive TCRγδ cells. These retained cells could support engraftment, reduce risk of rejection, reduce the risk of infections, and expedite immune reconstitution, which is currently one of the major challenges after allogeneic and especially haploidentical stem cell transplantation.

2.5.1.3. Reduced rate of infections

A high rate of functional immune cells such as NK cells in addition to some TCRαβ cells will be transfused with the graft in sufficient numbers. Thus, a reduced rate of infections is expected when compared to historical data from studies, in which CD34+ enriched and total CD3+ T cell depleted grafts were used. Different from CD3/CD19 depleted grafts, the transplant of CD3 yd T cells has an additional protrective effect from viral and fungal infections.

2.5.1.4. Prevention of PTLD

The concurrent depletion of B cells provides a preventive measure against the development of PTLD.

2.5.2. Potential study specific risks for recipients

Recipients of allogeneic transplants are subject to risks from transplant related procedures and medication utilized. These major risks are independent of the TCRαβ/CD19 cell depletion using the Miltenyi CliniMACS® or the Prodigy® TCRαβ/CD19 systems and are laid out in the later part of this chapter. It is expected that administration of TCRαβ/CD19 depleted grafts might have positive effects on these risks.
Additional, study specific risks due to the depletion of TCRαβ/CD19 cells are as follows:

2.5.2.1. Graft versus-host disease

Incomplete or insufficient removal of TCRαβ alloreactive cells might result in potentially life threatening GVHD, acute or chronic. Measuring of residual TCRαβ T cells is therefore of great importance and has been implemented in the graft release criteria.
Additionally, incidence and severity of acute and chronic GVHD will be monitored continuously throughout the study and is part of the composite endpoint to assess patients' safety. Each case of aGVHD grade III–IV or cGVHD has to be confirmed via biopsy of the affected organ(s) and will be announced to the DSMB.

2.5.2.2. Viral and fungal infections

The major risk of a depletion of TCRαβ T cells during a haploidentical HSCT are invasive viral and fungal infections that can seriously threaten the outcome of an HSCT. Among viral infections most commonly cytomegalovirus (CMV), adenovirus (ADV), influenza/para-influenza, human herpes virus 6 (HHV6), BK virus and others are the causative agents and must be monitored meticulously through the trial in order to relpy swiftly with antiviral agents. More recently the availability of virus specific T cells improved the outcome of viral reactivation and disease, if applied in time. The major threat from fungal infections is an invasive aspergillus infection although fungal infections can be tackled efficiently with fungal prophylaxis and the availability of several effective anti-fungal agents.

2.5.2.3. Lymphoproliferative syndrome (LPS, PTLD)

Incomplete or insufficient removal of CD19+ B cells might result in PTLD, which could also be life threatening. This risk is not different from T replete or unmanipulated transplants, where no routine B cell removal is carried out. Nevertheless, PCR analysis for EBV infection/reactivation will be performed.

2.5.2.4. Potential sensitization to murine proteins

Patients could become sensitized against murine proteins, which are used during the cell separation process. Although the procedure is based on the depletion of the cells that are marked with the respective mouse-anti-CD19 and mouse-anti-biotin antibodies it cannot be excluded that a few marked cells will be infused. If the recipient has a pre-existing allergy, he or she may be at risk of allergic reactions during infusion of the cell graft. The residual amount of murine protein in the final product, however, is very low due to the depletion procedure (estimated maximum dose of 30 µg for a patient with 50 kg body weight). To date, no allergic reactions have been reported in patients receiving enriched cells processed with the Miltenyi systems. Thus, this risk is considered to be low due to the longstanding experience with the reagents in different selection techniques (i.e. CD34 selection: no clinically relevant sensitization has been reported since entering the market in 1997). The most severe potential risk, GVHD, is addressed in this trial through the release specifications and continuous monitoring of the patient with the possibility to intervene if GVHD ensues. Neither PTLD nor murine protein sensitization are considered a risk that would prohibit this clinical trial.

2.5.3. Discussion of the risk/benefit assessment and conclusion

Allogeneic HSCT and especially the transplantation of a haploidentical stem cell graft, carries substantial, well known risks that have to be weighed against the risk of the malignancy or underlying non-malignant disease as well as the consideration of other treatment options. For patients entering this study, an allogeneic HSCT has been deemed necessary by the treating physician and due to unavailability of a MSD, haploidentical HSCT is considered.
Disease indication allowed to enter this trial, moderate and severe SCD, is severe and have a poor long-term outcome if transplantation is not performed. The transplantation of TCRαβ and CD19 depleted haploidentical stem cell grafts offers considerable potential benefits. Due to the efficient TCRαβ cell depletion similar GVHD rates are expected as observed with $CD34^+$ selected or CD3/19 depleted grafts. However, GVHD as the most severe potential risk is addressed in this trial through the release specifications and continuous monitoring of the patient with the possibility to intervene if GVHD ensues. Neither PTLD nor murine protein sensitization are considered a risk that would prohibit this clinical trial. Eventually viral infections remain the major threat but do not differ from haploidentical HSCT for other indications. An experienced team and a meticulous monitoring for viral infections with a prompt implementation of adequate anti-viral therapy can abolish the majority of viral complications.
Benefits of this regimen are expected to be mediated by beneficial effector cells which are retained in the graft during the depletion procedure. These cells are expected to facilitate engraftment, reduce the risk of infections, and improve immune reconstitution. Especially the latter is currently one of the major challenges after HSCT. Additionally, these beneficial cells offer the advantage of using dose- and toxicity-reduced conditioning regimens and the efficient and selective depletion of TCRαβ cells allows the reduction of immunosuppressants post-HSCT for GVHD prophylaxis. In summary, this is expected to translate into a reduced treatment-related toxicity. Preliminary data from transplantation of patients with SCD treated during the pilot phase, preceding this phase II trial, with TCRαβ and CD19 depleted haploidentical stem cell grafts show a low rate of acute and chronic GVHD as well as high engraftment rates and a fast recovery of the immune system compared to data published for patients transplanted with CD34+ selected or CD3/CD19-depleted hematopoietic stem cell grafts. Therefore, overall, the available information suggests that the present study has a favorable risk-benefit ratio.
Additionally, 13 patients with advanced stage SCD were transplanted using a MSD according to the trial protocol, including patients above 15 years. This was the cut-off age for reduced outcome parameters such as OS, DFS and cGVHD of 88%, 81% and 20%, as reported by Cappelli et al (Cappelli, Hematologica, 2019). These outcome parameters for the MSD population in the pilot series were 100%, 100% and 0% for ext. cGVHD, so that even patients with a MSD could benefit from enrolment in this trial.

2.5.4. Major risks of allogeneic hematopoetic stem cell transplantation independent of a T cell depleted product

Major risks specific to allogeneic HSCT for the recipients, which are independent of the Miltenyi CliniMACS® or the Prodigy® TCRαβ/CD19 systems, are described in the following sections. It is expected that administration of TCRαβ/CD19 depleted grafts might have positive effects on these risks.

2.5.4.1. Conditioning and immune therapy

Conditioning and immune therapy used for patient preparation prior to HSCT of PBSC are associated with considerable toxicity. General treatment-related advers effects and expected complications of HSCT should be well known to experienced transplant physicians and are handled according to the institutional guidelines. Trial specific and for endpoint analysis relevant treatment related complications such as GVHD and rejection are described in more detail in *section 5.1.4.4*.

2.5.4.2. Graft infusion

Symptoms may include changes in heart rate, arrhythmia, changes in blood pressure, fever, chills, sweats, nausea, vomiting, diarrhea, abdominal cramping, hemoglobinuria, acute renal failure, allergic reactions, respiratory dysfunction, or headache. These symptoms are rare, controllable and

independent of the haploidentical HSCT procedure.

2.5.4.3. Infections

Due to the underlying disease and to risks associated with immune suppression during conditioning, transplantation generally puts the patient at higher risk for potentially life-threatening bacterial, viral, or fungal infections. These risks, in particular the risk for viral reactivations and infections are potentially higher with T-cell depleted cell grafts (see above), which are mandatory in haploidentical transplantation. However, in this study a high rate of functional immune cells such as NK cells and TCRαβ cells will be transfused with the graft. Thus, a reduced rate of infections is expected when compared to historical data from studies, in which $CD34^+$-enriched and total $CD3^+$ T-cell depleted grafts were used. Nevertheless, as prophylactic measures patients will be closely monitored for signs of infections and will receive preemptive treatment as per institutional guidelines. With regard to bacterial infections the time to neutrophil engraftment is known to be significantly shorter compared to MSD BM grafts, so that this might be a beneficial effect for haploidentically transplanted patients. In the SCD patients treated during the pilot phase the time to engraftment was 18 versus 27 days for the haploidentical and MSD group, respectively [29].

2.5.4.4. Graft failure (GF) / Poor marrow function

Generally, T cell depletion of donor cells is associated with an increased incidence of GF or graft rejection in allogeneic transplant recipients. After allogeneic transplantation, the recipient's marrow function may be poor and leukopenia, anemia, or thrombocytopenia may result. GF may result in death if not reversed.

In the present study, however, selectively depleted cell grafts will be used, which still contain a high number of functional immune cells. Preliminary results in single patients have shown a remarkably fast functional reconstitution and good engraftment rates after transplantation of the TCRαβ and CD19 depleted grafts. In the SCD patients treated during the pilot phase no GF or rejection occurred [29].

Therefore, a lower rate of GFs and severely impaired marrow function after transplantation is expected compared to previous studies of haploidentical PBSC transplantation using differently processed cell grafts. Additionally, for each patient in the experimental arm (haploidentical HSCT) an autologous BM back-up will be generated and stored for eventual GF and graft rejection.
In case of suspected GF, the national coordinating investigator or the respective leading investigator will have to be consulted for advice regarding treatment.

2.5.4.5. Graft-versus-host disease

Potentially disabling acute or chronic GVHD may develop after allogeneic HSCT and can lead to death. GVHD is thought to be mainly initiated by TCRαβ cells contained in the PBSC graft. Selective depletion of the graft of TCRαβ cells thus is expected to reduce the incidence of severe GVHD. Still, GVHD is possible in the setting of the present study. See *section 5.1.4.4*. for treatment according to this protocol. In this study incidence and severity of acute and chronic GVHD will be monitored continuously throughout the study to assess patients' safety. Each case of aGVHD grade III–IV or cGVHD has to be confirmed by biopsy and will be announced to the DSMB.

2.5.4.6. Veno-occlusive disease (VOD) / Sinusoidal obstruction syndrome (SOS) of the liver

VOD/SOS is a manifestation of damage of the liver which can be caused by the conditioning regimens needed in haploidentical HSCT. It usually develops within 20 days after allogeneic transplantation and diagnosed according to the pediatric and adult EBMT criteria (see *appendix B.5*).
Recipients developing VOD will have to be monitored closely and will receive appropriate supportive care and therapy (e.g. Defibrotide) including careful fluid management according to international guidelines. In case of suspected VOD/SOS, the national coordinating investigator or the respective leading investigator should be consulted for advice regarding management.

2.5.4.7. Post-HSCT neurotoxicity and posterior reversible encephalopathy syndrome (PRES) in sickle cell disease

Neurotoxicity (NT) contributes significantly to HSCT-associated morbidity and mortality. Almost two thirds of the neurologic episodes (63%) occur within 100 days after transplantation [63]. Severe NT (posterior reversible encephalopathy syndrome (PRES), seizures, visual disturbance, cortical blindness, aphasia or ataxia) occurs in 4% - 11%. In particular in patients with hemoglobinopathies (thalassemia, SCD), the incidence is as high as 30%. The most frequently observed early post-transplant complication in SCD is PRES, with an incidence of up to 25% [64].
Major triggers for NT are the calcineurin inhibitors (CNI) such as cyclosporine A (CsA) and FK506. The higher incidence of NT in hemoglobinopathies might be due to impaired hepatic metabolization of CsA and FK506 based on iron overload [65]. However, also infusion modalities of CNI might impact on NT, since it was more frequently reported after 4-hours bolus injections (10.3%) than continuous infusion (3.3%) of FK506 [66].
Significant risk factors for neurologic complications are allo-HSCT, unrelated donors, and high-grade (>grade 2) aGVHD (Kang et al, Biol Blood Marrow Transplant (2015). In a recent post-HSCT study with children hypertension, any grade of aGVHD and hemoglobinopathies were significantly associated with the development of PRES [65, 67-69].
In fact, PRES is highly prevalent in SCD patients, independently of HSCT, also compared to thalassemia [63, 65, 68, 69]. Most importantly, the excessive NT observed in SCD might also be related to the systemic vasculopathy [70-72]. This is suggestive for the assumption that PRES belongs to the group of post-HSCT endothelial complications such as VOD, CLS, TAM, ES, aGVHD and others; a hypothesis supported by the observation from a retrospective analysis of 200 HSCT where an initial frequency rate of 38% of VOD (from 1995 to 2000) decreased up to 8% (2007-2013) after the introduction of FK506, with no more cases of VOD observed after the switch in GVHD prophylaxis to FK506.
In our cohort, NT was mostly observed in patients with older age and exposure to pro-inflammatory triggers such as T-repleted allo-HSCT (MSD and MUD). The incidence of NT correlated with that of aGVHD (4/5 patients with severe NT experienced aGVHD compared to an overall GVHD rate of 29%).
No comparative studies have been conducted so far to examine NT in HSCT recipients with regard to CNIs (CsA versus FK506), but preliminary data are indicative for a less neurotoxic effect of FK506 when administered as continuous infusion (80% of NT events under CsA prophylaxis versus 20% under FK506) (Kleinschmidt et al, Blood 2018, Suppl.)
Based on these data, the immunosuppressive therapy for the protocol will consist of FK506 as continuous infusion over 20 hours. The starting dose will be 0.03 mg/kg/day; further dosage will be adjusted to a serum level target of 5 - 8 ng/ml.
Cerebral MRI is the key investigation for the diagnosis of PRES. MRI is superior to CT for the diagnosis of PRES but CT may be easier to obtain first. CT findings are often normal or nonspecific. The characteristic neuroimaging is a hyperintense signal, distributing in the parietal and occipital lobes on FLAIR images. The non-specific clinical manifestations and multiplicity of radiological patterns raise diagnostic challenges [73].
Contrary to its name, PRES is not always 'reversible', and delay of diagnosis and therapy may cause secondary complications such as status epilepticus, intracranial hemorrhage, ischemic infarction, resulting in permanent neurological deficits or death [74].
The therapeutic mainstay is prophylactic use of antiepileptic medication as recommended in this trial, adequate anti-hypertensive management and adjustment of serum drug levels or in case of failure replacement of calcineurin inhibitors with everolimus (mTOR Inhibitor).
In case of suspected PRES, the national coordinating investigator or the respective leading investigator will have to be consulted for advice regarding management.

2.5.4.8. Engraftment syndrome

Presentation of an engraftment syndrome or capillary leak syndrome during engraftment consists of fever, skin rash, pulmonary edema, hypoxia and increase of C-reactive protein (CRP) values, often accompanied by weight gain, rising creatinine, hepatic dysfunction, diarrhea and encephalopathy (EBMT Handboook, 2019, 42.3.3) and usually heralds neutrophil engraftment, preceeding it by a couple of days. The pathophysiology seems to be that of a classical post-HSCT endotheliopathy triggered by a

massive release of pro-inflammatory cytokines. In patients with SCD a variation of this clinical presentation is observed in approximately 40% of SCD patients, depending on the patient population being transplanted (adult/advanced stage SCD compared to children). These patients experience pain episodes around the time of stem cell engraftment, heralding engraftment by approximately 8-10 days, similar to classical engraftment syndrome. The 'engraftment pain' after HSCT is similar to the experience of pain crises, well known to SCD patients. The cause is unknown, but it can be speculated that the same inflammatory storm adds a substantial painful irritation to the pre-damaged endothelium. It seems that SCD related engraftment syndrome is more prominent in advanced stage SCD patients with multiple osteonecrotic leasons undergoing a haploidentical HSCT where this engraftment related phenomenon of cytokine release is potentiated by the substantially higher number of transplanted stem cells. The pain episodes can be crucial and often refractory, requiring high-dose and prolonged analgetic therapy. Even opioids sometimes cannot resolve engraftment pain satisfactorily so that additional medications need to be added (see below).
In case of suspected engraftment pain, refractory to institutional standards, the national coordinating investigator or the respective leading investigator can be consulted for advice regarding management of uncontrollable pain episodes.

2.5.4.9. Death

After haploidentical HSCT, patients have a considerable risk of treatment related mortality within the first year after transplantation (depending on the study, between 20% and 50%, [16, 54]. This results from severe regimen related toxicity, GVHD, risks of hemorrhage, opportunistic infections, or other transplant-related complications. The mortality in the pilot population was 12% and therefore significantly lower compared to the published registry data. In this study, incidence and severity of TRM will be monitored continuously throughout the study to assess patient safety. Each case of TRM until Day 100 post HSCT has to be reported immediately and will be reported to the DSMB.

3. STUDY OBJECTIVES AND ENDPOINTS

3.1. Objectives

3.1.1. Primary objectives

The objective of this phase II trial is to prove that EFS following T-Haplo-SCT (experimental group E) is non-inferior to a MSD HSCT (reference group R) as well as evaluation of safety/tolerability and feasibility of haploidentical PBSC grafts depleted of TCR$\alpha\beta$ and CD19+ cells using the Miltenyi CliniMACS® or the Prodigy® TCR$\alpha\beta$/CD19 systems in adult and paediatric patients with SCD.

Event free survival (EFS), measured from HSCT until event, is defined as:

Acute graft-versus-host disease grade III–IV:
GVHD occurring within 100 days after SCT.

Severity graded according to MAGIC criteria (*appendix B.1*):

- Incidence of aGVHD grades III–IV
- Time until occurrence of aGVHD grades III–IV

Primary graft failure (pGF):

- ANC <0.5 × 10^9/L by Day 28
- Platelets <20 × 10^9/L

(Hemoglobin <8 g/dL Is omitted due to inclusion of donors with SCD heterozygosity)
and/or disease recurrence

Death (from any reason)

Moderate and severe chronic graft-versus-host diseases (cGVHD):
Incidence/severity graded according to the NIH Consensus Guidelines fom 2015 [17] (*appendix B.2*)

3.1.2. Secondary objectives

Secondary objectives are devided in key secondary objectives and secondary objectives:

Key secondary objectives:

- Overall survival (OS)
- Secondary graft failure (sGF), defined as initial neutrophil engraftment followed by a decline in ANC <500/µl, unresponsive to growth factor therapy and/or other intervention
- Immune reconstitution
- Quality of Life Assessment (QoL) using EQ-5D (adult patients ≥18 years), PedsQL 4.0/ PedsQL SCD and SCT modules (paediatric patients, age <18 years) and FACT-BMT (adult patients ≥18 years) at baseline, Day 100, 180 and after 1 and 2 years
- Fertility
- Disease free survivial (DFS)

Secondary objectives:

- Incidence of TRM at all visits throughout the study
- Incidence of grade I-II aGVHD until Day 100 post-HSCT
- Incidence and severity of cGVHD 1 year after omission of immunosuppression
- Incidence and severity of acute infusional toxicities
- Incidence and severity of transplant-related neurotoxicity and PRES

Feasibility outcome variables

- Neutrophil and platelet engraftment from Day 0 to Day 28 defined as
  - Neutrophil engraftment: ANC cell counts and time to reach >500 neutrophils/µl for three consecutive days
  - Platelet engraftment: platelet counts and time to reach ≥20.000 platelets/µl for 3 consecutive days with independence from platelet transfusions for 7 days
- Transfusion requirement from Day 0 to Day 100 defined as
  - Number of transfusions (thrombocytes, erythrocytes and other blood products)
  - Time to last transfusion of thrombocytes, erythrocytes and other blood products
- Days of (re)hospitalization assessed at Day 28, Day 100 and after 1 and 2 years

Laboratory outcome parameters

- Donor chimerism by
  - PCR-analysis of peripheral blood samples collected weekly starting with the day of engraftment until day 100, biweekly until day 180 followed by monthly until 12 months post-HSCT compared to samples from donor and recipient collected prior to HSCT (excluded from this procedure is the time around weaning of immunosuppression and patient presenting with a mixed chimerism <90%)
  - PCR-analysis of bone marrow samples collected on Day 100 post-HSCT and Day 240 if indicated by persistent mixed chimerism (see below *section 5.1.4.4.7*)
- Split chimerism, assessed centrally by PCR -analysis of peripheral blood and/or bone marrow samples (if needed) collected only if full donor chimerism drops <90%
- Weekly immune cell phenotyping (CD3, CD19, CD56, CD4, CD8) is recommended in the early post-HSCT period up Day 180 (*appendix B.9*).
- Immunesystem reconstitution, assessed locally as baseline **before** start of conditioning and then monthly starting with engraftment monthly until 1 year post-HSCT. Additional 3-monthly immune monitoring should be considered if immune reconstitution is not completed by 12 months post-HSCT (*appendix B.9*).
  Centralized immune system reconstitution is required at baseline before start of conditioning and at

Day 60, 100 and after 1 and 2 years (*appendix B.9*).

3.1.3. Safety objectives

- Incidence and type of infections
  - Incidence of CMV, ADV, EBV and aspergillus infections as well as other viral, bacterial and fungal infections at Day 100 and 1 year and 2 years post-HSCT
  - Number of virus reactivations of CMV, ADV, EBV, HHV6, BK virus by PCR weekly until Day 100, followed by every other week until cessation of immunosuppression and no clinical signs of GVHD. Continue in case of viral reactivation and/or immunosuppression for treatment of GVHD.
  - Aspergillus by PCR when expected clinically, followed weekly until complete resolution
- Incidence, severity and type of AE and SAE, clinically relevant vital signs and safety laboratory parameters
  - SAE throughout the study
  - Known therapy-related toxicity as AE from Day -12 to Day 0 (during conditioning and prior to stem cell transplantation)
  - All AE from Day 0 until 24 months post-HSCT
- Vital signs and physical examination throughout the study
- Safety laboratory parameters (clinical chemistry and complete blood counts) at baseline and from Day -4 to Day 100
- Concomitant medication
  - Concomitant medication from baseline to Day 100
  - Documentation of new treatment with cellular products (erythrocytes, thrombocytes or virus-specific T cells, VST) after Day 100
- Assessment of fertility via imaging and laboratory parameters before HSCTand at several timepoints post-HSCT as indicated below

3.2. Study hypothesis

- Equal or reduced treatment-related morbidity and mortality, due to the lower toxicity of the conditioning regimen and a lower rate of severe infections
- Equal or improved engraftment
- Equal or lower incidence of rejection
- Equal or improved immune reconstitution (increased speed, spectrum, functionality of immune system reconstitution)
- Equal or lower incidence of severe viral infections
- Equal or lower incidence of aGVHD and cGVHD
- Equal or overall improved QoL
- Equal or reduced incidence of SCD-related complications
- Equal or improvement of SCD-related vasculopathy (TCD, renal function, cerebral imaging, neurocognitive testing)
- Reduction of transplant-related infertility by use of treosulfan

4. STUDY DESIGN

4.1. Study overview

This multi-center, open-label, phase II clinical trial will assess safety/tolerability and feasibility of an HSCT with haploidentical peripheral blood stem cell grafts depleted of TCRαβ and CD19 cells using the Miltenyi CliniMACS® or the Prodigy® TCR$\alpha\beta$/CD19 systems in adult and pediatric patients suffering from SCD. Patients will be prepared for transplantation by a T cell depletion regimen prior to HSCT. The

avoidance of rejection and in particular GVHD is pivotal. Therefore, for all patients GVHD prophylaxis is applied. Safety will be primarily assessed by determining occurrence and time to aGVHD grade III–IV at Day 100 post-transplantation and cGVHD at 1 and 2 years post-HSCT. Secondary endpoints are OS, DFS, GF, immune reconstitution (T, B and NK cell subsets as well as T regulatory cells), quality of life and fertility up to 2 years post-HSCT.

Patients who fulfill inclusion criteria will be stratified according to donor availability. Next to the pre-transplant investigations and exams, centres should:

- obtain an **autologous bone marrow back-up** from each patient in the experimental arm (haploidentical HSCT)
- discuss and perform **fertility preservation** means as indicated below
- place a silicon central venous line device with subcutaneous tunneling (Hickman, Broviac et al) or any other form of central venous access according to institutional guidelines

Prior to any interventional procedure, an exchange transfusion is strongly recommended to reduce the HbS fraction to below 30% in order to avoid any sickle related crisis during these invasive procedures.

The conditioning regimen for both arms is identical with the exception of the timing of the immunotherapy.

Immunotherapy consists of ATG from Neovii (Grafalon®) which is used due to the shorter half-life compared to the ATG product from Sanofi (Thymoglobuline®).

- For T-depleted haploidentical grafts it is dosed at **15mg/kg**, given upfront prior to the start of chemotherapy on day -10 to -8,
- For MSD, the dose is **10mg/kg** on day -3 to -1 immediately prior to BM infusion.

Chemotherapy, applied between days -10 and -2 consists of

- Thiotepa 2 x 5 mg/kg
- Fludarabine 4 x 40 mg/m^2
- Treosulfan 3 x 14 g/m^2

This conditioning regimen is well established and safe, myeloablative and highly immunosuppressive and was applied in more than 40 SCD patients during the pilot phase for this trial. In contrast to busulfan, treosulfan is well tolerated, does not pass the blood-brain barrier and has a lower incidence of endothelial complications such as sinusoidal obstruction syndrome (VOD/SOS).

Grafts:

Haploidentical grafts will be engineered via Clinimax® or Prodigy® with a content of CD34 >1 x 10^7/kg and CD3 aß <5x10^4/kg.

In BM grafts from MSD, the CD34 count should range between 2-8 x 10^6/kg.

For detailed specifications of the graft, see below.

The duration of treatment is standard, consisting of approximately 10 days of conditioning and stem cell infusion, followed by an in-patient follow-up of approximately 30 days.

Post-Transplant Immunosuppression:

Post-transplant immunosuppression is a pivotal part for a successful GVHD-free and rejection free HSCT in hemoglobinopathies, in particular with haploidentical T-depleted grafts. In contrast to HSCT in malignant diseases it fulfills the task of GVHD prevention mainly in the MSD arm and prevention of graft rejection in the T depleted haploidentical arm of the trial.

The **post-HSCT immunosuppressive therapy** consists of

- Mofetil mycophenolate (MMF, ie. Cellcept®) at 4 x 300mg/m^2 BSA starting at day -1
- Tacrolimus (Prograf®) at 0.03mg/KG BW continuous infusion over 20 hours starting at day -3, with a targeted serum level range of 5ng/ml to 8 ng/ml

For the duration of immunosuppression see *section 5.1.4.2.2*.

Discharge criteria from inpatient care are:

- Transfusion independence
- Appropriate oral intake
- Successful oralization of immunosuppressive and supportive medication
- No acute transplant related complications, no GVHD
- Stable or rising chimerism >80% at 2 consecutive weekly timepoints
- Stable tacrolimus serum levels after oralization (between 5ng/ml and 8ng/ml)

Total **follow-up** for this trial is 1 year after the omission of immunesuppression (usually 2 years total) which allows assessment of all trial relevant parameters, in particular the primary composite endpoint of GVHD-free/DFS survival.

Patients will then be followed according to institutional guidelines and legal requirements.

Fig. 1: Study design and individual patient timeline

4.2. Rationale for study design

For the following reasons no prospective, randomized study design for the present study has been chosen. A MSD HSCT is standard of care and serves as the reference arm. Since donor availability is limited to <20% a randomized trial would have not been able to recruit enough patients in due time. Furthermore, patients with no MSD (>80% of all SCD patients and the target population of the experimental arm), would have been excluded from such a randomized trial design.
Therefore, a natural randomization process via donor availability was preferred and patients are assigned accordingly. Following these arguments, the conduct of a non-randomized, prospective study was chosen to determine safety and tolerability of TCRαβ and CD19 depleted PBSC grafts in the haploidentical setting for SCD.
The study will be performed as a multi-center trial to reach the calculated sample size in the planned time period.

4.3. Study population

Both, pediatric and adult patients from >1 year up to 35 years of age will be included in this trial. It is planned to enroll 212 patients overall with the following genotypes: HgB S/S, HgB S/C or S 0/+.

4.3.1. Selection criteria

Patient Inclusion Criteria

A patient has to meet all of the following criteria to be eligible:

- Age 1yr to 35yrs
- Homozygous hemoglobin S disease or heterozygous hemoglobin SC or S 0/+
- Study specific consent given
- Preexisting severe or moderate SCD related complications:
  - Clinically significant neurological event (stroke) or deficit
  - Silent crisis, neurocognitive deficit
  - Pathological angio-MRI with TOF Sequence
  - TCD velocity >200 cm/s at 2 occasions >1 month apart
  - More than **5** vaso-occlusive crises (VOC) in the past 1 year **or** more than **20** VOC in a lifetime

- Two or more episodes of acute chest syndrome (ACS) in their lifetime or one episode of ACS in the past 24 months
- Chronic transfusion requirement **or** more than 8 transfusions **or** one exchange transfusion in a lifetime
- Transfusion-refractory allo-immunization
- More than five SCD-related hospitalizations in a lifetime
- Beginning pulmonary hypertension
- Osteonecrosis at more than 2 sites
- Beginning SCD Nephropathy
- Recurrent priapism (>2)

Patient Exclusion Criteria

- Karnofsky or Lansky Performance Score < 70%
- Patients with donor-specific antibodies (DSA) against the potential stem cell donor by either
  - Cell-based crossmatched assays (Complement-dependent cytotoxicity; CDC) or
  - Flow cytometry crossmatch test or
  - Solid-phase immunoassays (SPI) or
  - Modified SPI such as C4d and C1q assays

  Whichever method the participating center is experienced in.
- Patients with major ABO incompatibility defined according to EBMT Handbook, Edition 2019 Tab 23.1.:

ABO incompatibility	Recipient	Donor
	0	A
	0	B
Major	0	AB
	A	AB
	B	AB

- **Cardiac function:**
  - Ejection fraction at rest <45.0% on echocardiography or
  - Shortening fraction of ≥ 27.0% by echocardiogram or radionuclide scan (MUGA)
  - Patients with > grade II hypertension by CommonToxicity Criteria (CTC)
- **Renal function:**
  - Estimated creatinine clearance (for patients > 12 years) less than 50.0 mL/minute
  - for pediatric patients (> 1 year to 12 years), GFR estimated by the updated Schwartz formula < 90.0 mL/min/1.73 m2. If < 90 mL/min/1.73 m2, renal function must be measured by 24-hour creatinine clearance or nuclear GFR and must be > 70.0 mL/min/1.73 m2 or
  - Creatinine clearance below threshold defined for stem cell transplantation according to local clinical standard
- **Pulmonary function:**
  - DLCO >50% (adjusted for hemoglobin), and FVC and FEV1≥50%; children unable to perform for PFTs, O2 saturation <92% on room air.
- **Liver function:**
  - Total bilirubin > 3 x the upper limit of normal (unless elevated bilirubin is attributed to Gilbert's Syndrome et al) and ALT/AST > 2.5x the upper limit of normal.
  - Chronic active viral hepatitis
- Women who are pregnant (positive serum or urine βHCG) or breastfeeding
  Note: Women of childbearing potential must have a negative serum pregnancy test at study entry.
- Adults of reproductive potential not willing to use an effective method of birth control during study treatment and for at least 12 months thereafter,

- History of uncontrolled autoimmune disease or on active treatment
- Patient unable to comply with the treatment protocol
- Prior autologous or allogeneic hematopoietic stem cell transplant
- Vaccination with a live virus vaccine during the trial
- HIV infection
- Patients with a history of psychiatric illness or a condition which could interfere with their ability to understand the requirements of the study (this includes alcoholism/drug addiction)
- Patients unwilling or unable to comply with the protocol or unable to give informed consent.
- Concurrent severe or uncontrolled medical disease (e.g. uncontrolled diabetes, congestive heart failure, myocardial infarction within 6 months prior to the study, unstable and uncontrolled hypertension, chronic renal disease, or active uncontrolled infection) which by assessment of the treating physician could compromise participation in the study.
- Patients with prior malignancies, except resected non-melanoma or treated cervical carcinoma in situ. Cancer treated with curative intent >5 years previously will be allowed. Cancer treated with curative intent < 5 years previously will not be allowed unless approved by the Protocol Officer or one of the Protocol Chairs.

Donor Selection

1. Haploidentical family member previously identified as eligible donor by donor/recipient cross-matching including HLA-typing for the experimental arm. 10/10 MSD for the reference arm according to international and institutional guidelines.
2. Donor age preferably is ≥16 years, but younger are also eligible according to institutional guidelines.
   Note: Evaluation for allogeneic hematopoietic cell donation has to performed at the collection center according to local standard practice. Also informed consent for mobilization and collection of peripheral blood HSC or in case of a MSD consent for BM collection according to institutional guidelines has to have been given in this context independently of the present clinical study. Stem cell mobilization and collection procedures as well as bone marrow harvest are not part of this study and will be performed at the participating center according to local standard procedures.
   MSD donors are selected and managed according to international regulations and guidelines as well as local institutional regulations.
3. Donor selection hierarchy:
   a. for haploidentical donors (experimental arm):
      i. No DSA in any validated test (chapter 5.2.2.1.)
      ii. No major AB0 incompatibility (as indicated below)
      iii. CMV donor recipient match
      iv. Donor age (younger>older)
      v. Donor sex (male>female)
   b. For MSD (reference arm)*
      i. HLA compatibility, with 10/10
      ii. CMV serological status of positive donors in case of positive recipients
      iii. BM as stem cell source
      iv. Donor age, being preferable a younger donor
      v. Donor gender, with a male donor preferred, particularly for a male recipient
      vi. ABO major compatibility
      vii. Donor center location
      viii. ABO minor compatibility (unpublished data)

   ** EBMT Handbook, Edition 2019, chapter 12.4.6*
4. Study specific informed consent given.

4.3.2. Withdrawal and replacement

Patient Withdrawal and Replacement

Patients or their legal representatives are free to withdraw consent to participate in the study at any time without penalty and without stating any reason. This will not prejudice the future medical care of the patient in any way. All patients have to be closely monitored and treated as appropriate, according to the respective guidelines for patients with SCD. The investigators are authorized to perform additional follow-up examinations at their discretion.

Patients have to be withdrawn if any of the following events occur:

- The patient withdraws consent
- Pregnancy
  If a patient has to be withdrawn because of pregnancy, she has to be followed- up until after the delivery of the child and reported to the sponsor accordingly.

The Sponsor or the investigators are free to withdraw patients when this is considered medically or otherwise necessary.

Justified reasons are:

- Adverse event(s)
- Violation of eligibility criteria
- Violation of the study protocol (e.g. conditioning or failure to attend study visits)
- Donor withdrawal

Reason(s) for and the justifications of the withdrawal always have to be documented on the electronic case report form (eCRF) in as much detail as possible. If a patient is prematurely withdrawn from the study for any reason, the investigator will under all circumstances try to perform all evaluations described for the early termination visit. Withdrawn patients can be replaced.
Screening failures and patients, who receive hematopoietic cell grafts with an insufficient number of $CD34^+CD45^+$ cells (<4.0 × 10^6/kg BW) may be replaced to include at least 212 evaluable patients into the study.
A drop-out rate of 5% is estimated for this study. Thus, 250 patients are assessed and to obtain complete, evaluable data sets of 212 patients approximately 223 (5% drop-out) patients will presumably be enrolled.

Trial Design

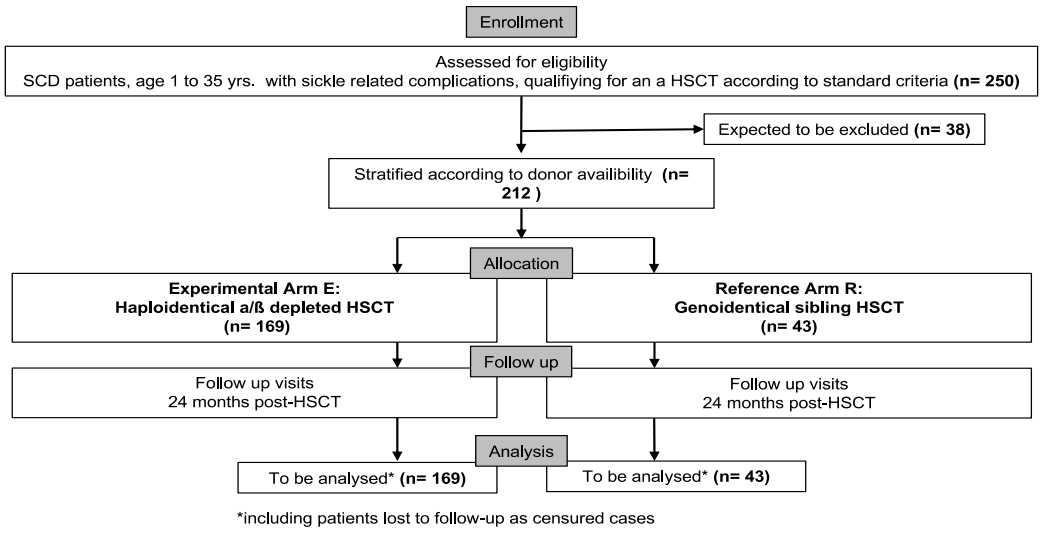


Fig. 2: Trial design for statistical analysis

Donor Replacement

If a patient withdraws study-specific consent prior to conditioning and transplantation the blood sample of the donor will be destroyed in case it has already been collected. Should a donor be no longer eligible for stem cell mobilization or apheresis according to the responsible collection center's judgement, the investigator will be informed immediately. Reasons have to be stated and will be documented on the electronic case report form (eCRF) of the recipient. An alternative donor for the patient should be identified as soon as possible. If this is not possible the patient has to be withdrawn from the study or in case of the loss of a MSD the patient can be enrolled in the experimental arm.

4.3.3. Patient identification and randomization

No randomization is planned for this open-label study.

Patients will be approached for this study after the decision to proceed with transplantation has been made, the patient will be stratified to either the reference or the experimental arm depending on the donor availability. Transplant physicians will evaluate the patient eligibility for assignment to this study. Only patients who comply with all entry (in- and exclusion) criteria will receive a patient identification number and will be assigned to the course of the study treatments. Eligibility criteria will be verified, and ineligible patients will proceed off-study and no further follow-up will be obtained.

Eligible patients willing to participate in the study will sign an approved consent form prior to any study relevant treatment.

Patient identification numbers (ID) will be assigned randomly and anonymized using the Mainzelliste. The Mainzelliste is a web-based first level pseudonymisation service. It allows for the creation of personal identifiers (PID) from identifying attributes (IDAT). The functions are available through a REST interface. By this means, data from each study subject is connectable between institutions, yet legal data protection requirements can be ensured through the implementation of power separations, including pseudonymised data storage.

This patient ID will be recorded in the electronic case report forms (eCRFs) and all study-specific documents of a specific patient and in the site patient file.

The investigator will keep a record with the names, the birth dates and the patient number of the patients (subject identification list) to allow checking of data in the clinical files of each patient, when required. This record will remain at the study site.

4.3.4. Protocol violations

Except in the case of a medical emergency, no protocol violation is authorized outside amendments established by the Sponsor and approved by an IEC/IRB (Institutional Review Board). Protocol violations may affect the conduct of the study from legal and ethical points of view and may influence the statistical analysis and pertinence of the study. In case of a medical emergency event in a patient leading to a protocol violation this will only be allowed for the single patient. The investigator has to contact the Sponsor to clarify if the patient may continue in the study.

Recurrent unexplained protocol violations can lead to exclusion of the centre.

4.3.5. Premature termination of the study

If the investigator, the Sponsor or the clinical monitor becomes aware of conditions or events suggesting a possible hazard to patients in case the study continues, the study may be terminated after appropriate consultation between the relevant parties. The study may also be terminated early at the Sponsor's discretion in the absence of such a finding.
Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the patients enrolled in the study
- Failure to enroll patients at an acceptable rate
- A decision on the part of the Sponsor to suspend or discontinue development of the haploidentical treatment concept for SCD

4.4. Study population

Patients in the experimental arm will be treated in this study with individual hematopoietic PBSC grafts from haploidentical donors depleted of TCR$\alpha\beta$ and CD19^{+} cells, alternatively the reference arm will receive a BM graft from a MSD. Prior to transplantation patients will be prepared with an almost identical conditioning regimen for both arms. Conditioning regimens for patients are described in more detail below.

4.4.1. Preparation of the hematopoietic stem cell graft (IMP)

Stem cell mobilization, stem cell apheresis and BM collection will be performed according to standard of care following legal requirements including, but not limited to the German transfusion law and relevant guidelines (ie Transfusionsgesetz in der Fassung der Bekanntmachung vom 28. August 2007 (BGBI. I S: 2169). In order to ensure that the TCRαβ and CD19-depleted stem cell graft is available for the patient at the required time, coordination of the parties concerned has to be performed according to clinical practice for haploidentical HSCT with manipulated stem cell grafts following internal guidelines and legal requirements (e.g. German guideline 'Richtlinie zur Transplantation peripherer Blutzellen'). It is the Investigator's responsibility to ensure that a suitable stem cell graft will be available for the patient in time.
The manufacturing process of the TCRαβ and CD19 depleted cell grafts will be performed in centralized laboratories assigned by the Sponsor (see *appendix A.3*). The manufacturing process and quality control will be performed according to validated procedures and documented in accordance with full GMP requirements.

The stem cell apheresis will be depleted of TCRαβ and CD19 cells by negative selection using the automated Miltenyi CliniMACS® or the Prodigy® TCR$\alpha\beta$/CD19 systems as described in the IMPD, the CliniMACS® and Prodigy® user manual, respectively and according to institutional Standard Operating Procedures (SOPs) in place and validated at the manufacturing sites.

The specification for the final formulation of the IMPs has been set according to findings from previous studies [33, 75-77].

For transplantation the following graft composition is targeted

- Minimum percentage of viable CD34+CD45+ cells ≥95%
- Number of viable CD34+CD45+ cells ≥1 × 10^7 cells/kg BW of the patient
- Number of TCRαβ+ cells ≤5 x 10^4 cells/kg BW of the patient
- Number of CD20+ cells ≤1 × 10^5 cells/kg BW of the patient

As noted above the target graft cell doses following processing with the Miltenyi CliniMACS® or the Prodigy® TCR$\alpha\beta$/CD19 systems are defined as both, a CD34+CD45+ cell count of ≥1 × 10^7 cells/kg BW of the recipient and a TCR$\alpha\beta$ cell count of ≤5 x 10^4 cells/kg BW of the recipient. It is not permitted to exceed the target cell number of 5 x 10^4 TCRαβ cells/kg BW. For any graft with TCRαβ cell content above this limit, a part of the graft will be retained to adjust such grafts to the maximal T cell content ≤5 x 10^4 TCRαβ cells/kg BW.

In that case falling below the limit of 1 × 10^7 CD34+CD45+ cells/kg BW of the patient is possible but must not fall below **5 x 10^6 CD34+CD45+ cells/kg BW**.

In order to reach the minimum limit of ≥5 × 10^6 CD34+CD45+ cells/kg BW up to additional two CD34+CD45+ cell separations can be performed following further apheresis procedures.

Since the potential result of stem cell mobilization varies with the age of the donor (more reliable result is expected with younger donors) processing of grafts depends on the age of the donor.

- In general, it is expected that in donors <50 years, grafts reach sufficient cell counts. Therefore, patient conditioning normally starts immediately upon enrolment and transplantation will be performed immediately after stem cell apheresis and depletion as described below.
- If poor mobilization is expected (e.g. in donors ≥50 years) the grafts may be cryopreserved prior to transplantation. Thus, a sufficient CD34+CD45+ cell count may be confirmed before the patient starts with conditioning. In this case individual grafts resulting from maximum three subsequent

cycles of stem cell apheresis and cell depletion will be cryopreserved. The patient will be allowed to enter into treatment when sufficient CD34+CD45+ cell counts have been confirmed by the subsequent analysis only.

It is up to the responsible investigator to decide case appropriate approach.
In case clarification is needed the national coordinating investigator or the Sponsor should be contacted.

All specification parameters for IMP release will be documented in the 'certificate of analysis' which will be provided to the study centers together with the IMP. Beside the numbers of CD34+CD45+-, TCRαβ and CD20 cells these are:

- Number of CD3+ cells (total and per kg BW of the patient)
- Number of CD45+ cells (total and per kg BW of the patient)
- The percentage of recovered viable CD45+ cells after TCR$\alpha\beta$ and CD19 depletion: target value ≥90%
- Hematocrit in mL/mL erythrocytes
- Result of visual control (bags undamaged, no cell aggregates visible).

Sterility will be asserted and documented but is not relevant for release, since the tests can be completed after the time of transplantation, only.

Log depletion of TCRαβ and CD19 cells and CD56+CD16+ cell counts for additional evaluation of the graft, which are not parameters of drug specification, will also be documented in the 'certificate of analysis Quality control will be monitored (see IMPD).

4.4.2. Packaging, labeling and storage

Packaging

The graft will be delivered in primary bags which are packed for transport in an outer package (sterile overwrap packaging). The graft will be packaged in Cryobags. Hard-case aluminum cassettes or equivalent packaging will be used for secondary packaging.

Labeling
The bags/packaging of the graft will be labeled in accordance with the applicable regulatory guidelines (for labeling details see IMPD and Investigators File). Labels will be in English.

Storage
The graft is intended for direct administration after completion of the preparation process with a shelf-life of 72 h calculated from end of apheresis, with storage at 4±2°C.
The graft can be stored in the vapor phase over liquid nitrogen at the manufacturing site (shelf-life of ≤1 year) and thawed at bedside for direct application of the cells according to procedures in clinical routine.

4.4.3. Transport of investigational product

The transport of the stem cell apheresis product to the GMP Manufacturing site will follow the local standard practice and SOPs developed by the GMP Manufacturing sites. The transport of the graft from the GMP Manufacturing site to the local clinical site for transplantation will follow the local standards of clinical practice for the transport of allogeneic blood stem cell transplants following legal requirements and relevant guidelines.

4.4.4. Administration of investigational product

For transplantation patients will receive the hematopoietic stem cell grafts intravenously on Day 0, Day 1 (and Day 2) as appropriate to reach the CD34+CD45+ target cell count according to the respective center's institutional guidelines for methods of infusion.

4.5. Compliance

The study treatment will be administered by the investigator. Therefore, patient compliance with study treatment will not be monitored.

Patients non-compliant with the study protocol (e.g. non-attendance at study visits or refusal to undergo certain assessments) may be excluded at the discretion of the investigator or sponsor.

4.6. Graft and device accountability

The investigator is responsible for maintaining accurate accountability records throughout the study. The administration of the cell grafts will be documented in the CRF and in the patient's medical file. The investigator is responsible for ensuring that all unused or partially used cell grafts will be disposed of according to the national legal regulations and the local regulations for biological products.

Any stem cell graft which will not be used during the study for transplantation will be stored in case it is needed for later treatment of the patient in agreement with the patient information and the informed consent. Upon death of the patient it will be destroyed. The manufacturer has to ensure, that disposal will follow the national legal regulations and all relevant regulations for biological products.

5. STUDY PROCEDURES

5.1. Study treatment plan

All patients with SCD enrolled in the study will undergo an HSCT from either a MSD if available or a haploidentical family donor. Haploidentical donors will have been treated for mobilization of hematopoietic HSC prior to PBSC collection by stem cell apheresis according to local standards at their collection center. Prior to infusion, the donor derived PBSC grafts will be depleted of TCRαβ positive and CD19 positive cells using the Miltenyi CliniMACS® or the Prodigy® TCR$\alpha\beta$/CD19 systems. HSCT from a MSD will be performed using a BM graft. Patients in both arms will undergo an almost identical myeloablative conditioning to enable engraftment of hematopoietic HSC prior to the intravenous infusion of the individual stem cell grafts. Patients will presumably be hospitalized for 6 weeks according to patient's clinical condition and/or institutional guidelines.

Beside the baseline visit, patients will have to attend regular follow-up visits up to the last follow-up visit at Day 720 post-transplantation.

Donors will have to attend the center for one visit to give their informed consent and for collection of blood samples for safety assessment and accompanying scientific programs. An additional follow-up visit for donors is scheduled 12 months after study termination.

A data safety monitoring board (DSMB) will be responsible for the overall safety of the patients in the study (for further details see *appendix A.5*). A continuous safety monitoring will be performed throughout the study for the parameters acute GVHD grade III–IV and TRM until Day 100 post transplantation. Statistical stopping guidelines have been defined to guarantee the patients' safety in the study and to allow for immediate reaction in case of elevated incidence rates.

5.1.1. Mobilization and collection of donor PBSC or BM

In this study, haploidentical family donors will donate peripheral blood HSC according to legal requirements and local institutional practice. Hematopoietic stem cell mobilization and collection of peripheral blood by stem cell apheresis will be performed according to relevant clinical and regulatory guidelines for collection and transplantation of PBSC grafts. Since HSCT is the only potentially curative therapeutic option for the critically ill patients in this study it is planned independently of this study. All procedures related to donors apart from an additional taking of a blood sample are therefore performed according to clinical routine and independent of this study.
The target stem cell dose is ≥1 × 10^7 CD34+CD45+ cells/kg BW of the recipient with ≥95% viable

CD34+CD45+ cells in the graft. Depending on the recipient's body weight, it is expected, that a total of mostly 2 stem cell apheresis runs of the donor will be needed to obtain the target number of CD34+CD45+ cells in the graft. In donors with poor mobilizing, in which the minimum CD34+CD45+ cell dose of ≥1 × 10^7 cells/kg BW is not achieved after 2 stem cell apheresis runs and the following TCR$\alpha\beta$ and CD19 depletion procedure, it is at the discretion of the transplant center to continue with a third stem cell apheresis run and PBSC collection.

BM will be collected according to institutional guidelines and legal requirements and is not part of this trial.

5.1.1.1. Poor mobilizer

Poor mobilizers are usually defined as patients with less than 2 × 10^6 CD34+ cells/kg collected or patients mobilizing less than 10–20 CD34+ cells/µl into the PB. Based on circulating CD34+CD45+ cells, it is possible to identify the following subgroups:

1. "borderline poor mobilizer" (11– 19 CD34+CD45+ cells/µL)
2. "relatively poor mobilizer" (6–10 CD34+CD45+ cells/µL)
3. "absolute poor mobilizer" (0–5 CD34+CD45+ cells/µL) [78]

Risk factors for poor mobilization are as follows (Adapted from Mohty et al, 2010):

- Age >60 years
- History of past chemotherapy
- Low or borderline peripheral blood cell counts
- Low CD34+ cell count before apheresis

In case of an expected "relative" or "absolute" poor mobilizer (<10/µl CD34+/CD45+ cells on day #4 of G-CSF stimulation) at the discretion of the local investigator and according to the institutional guidelines the use of plerixafor (Mozobil®, Genzyme; recommended dose 0.24 mg/kg/day SC) prior to the second day of mobilization can be considered.

5.1.2. Recipient preparation

5.1.2.1 Conditioning regimen

In most cases patient conditioning will be started prior to mobilization of the donor.

In case of an expected poor mobilizer (see *section 5.1.1.1.*) conditioning will be started after the graft has been collected and a sufficient $CD34^+CD45^+$ cell count has been confirmed (see *section 4.4.1.*). In this case the graft will be cryopreserved so that the time schedules of donor and recipient will be independent of each other. The donor will receive mobilization treatment and grafts will be prepared **before** the corresponding patient will be started on the conditioning.

Prior to conditioning patients should receive an **exchange transfusion** within **4 weeks** prior to start of conditioning (the closer the better) in order to avoid SCD related hemolysis and SCD crisis during central line placement, fertility preservation and conditioning. Fertility preservation according to international standards and institutional guidelines is highly recommended (see *section 10.2*).

Prior to transplantation, patients will receive a conditioning regimen consisting of fludarabine, thiotepa, treosulfan and ATG Neovii (Grafalon®) (table 1 and 2). Fludarabine, thiotepa and treosulfan are approved drugs which have been previously used in various conditioning regimens for allogeneic HCT. ATG Neovii (Grafalon®) is an approved drug for prophylaxis against solid organ transplant rejection and since 2011 in Germany approved for 'prophylaxis of graft-versus-host disease for unrelated stem cell transplant donors in adults.' In pediatric patients ATG Neovii (Grafalon®) will be used off-label in this protocol. **(see drug information in IB)**. ATG Neovii (Grafalon®) should be applied with anti-allergic prophylaxis with dimetindene and prednisolone (see *section 5.1.4.2.1.*).

Table 1: Conditioning regimen for MSD HSCT

Drug/Procedure	Day														
	-28 to -12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	+1	+2
Pre-HSCT Preparation															
Exchange Transfusion	X														
Fertility Preservation Procedures	X*+														
Central Venous Line Placement	X														
Isolation				X											
Conditioning															
Immunotherapy Grafalon® ATG 10mg/kg BW/day										X	X	X			
Thiotepa 2 x 5 mg/kg BW/day					X	X									
Fludarabine 40 mg/m² BSA/day						X	X	X	X						
Treosulfan 14g/m² BSA/day									X	X	X				
BM Transplantation													X		
Post-HSCT Immunotherapy															
Tacrolimus 0,03 mg/kg BW cont.infusion 20h										X	X	X	X	X	X
Mofetil Microphenolate 4 x 300 mg/m² BSA/day												X	X	X	X

* optional, + if indicated; if ovarian wedge resection or any other surgical procedure is indicated, this should be performed after the exchange transfusion but minimum a week prior to conditioning

Table 2: Conditioning regimen for haploidentical HSCT

Drug/Procedure	Day														
	-28 to -12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	+1	+2
Pre-HSCT Preparation															
Exchange Transfusion	X														
BM-Backup	X														
Fertility Preservation Procedures	X*+														
Central Venous Line Placement	X														
Isolation				X											
Conditioning															
Immunotherapy Grafalon® ATG **15**mg/kg BW/d			X	X	X										
Thiotepa 2 x 5 mg/kg BW/day					X	X									
Fludarabine 40 mg/m² BSA/day						X	X	X	X						
Treosulfan 14g/m² BSA/day									X	X	X				
Donor Preparation and Transplant															
Donor Stimulation								X	X	X	X	X	X*	X*	
PBSC Collection												X	X*	X*	
Graft Preparation													X	X*	X*
PBSC Transplantation													X	X*	X*
Post-HSCT Immunothetrapy															
Tacrolimus 0,03 mg/kg BW cont.infusion 20h										X	X	X	X	X	X
Mofetil Microphenolate 4 x 300 mg/m² BSA/day												X	X	X	X

*optional, if indicated; +if ovarian wedge resection or any other surgical procedure is indicated, this should be performed after the exchange transfusion but minimum a week prior to conditioning

5.1.3. PBSC transplantation

The individually manufactured IMP (released according to the IMPD) will be administered intravenously on Day 0 to all patients according to individual institutional guidelines after appropriate processing and quantification has been performed by the GMP manufacturing site. Additional transfusions on Day +1 (and Day 2) will be performed as necessary.
The number of transfusions will depend on the number of individual stem cell apheresis cycles needed to reach a sufficient content of ≥1 × 10^7/kg BW CD34+CD45+ cells for transplantation.
It has to be taken into consideration that the absolute CD3ab T cell count must not to exceed 5 x 10^4/kg BW.
Each individual graft may be administered either immediately after processing or may be cryopreserved after processing for subsequent single transfusion.
For prophylaxis of GVHD and rejection MMF and Tacrolimus has to be administration according to protocol (see *section 5.1.4.2.2.*).

5.1.4. Prophylaxis, supportive care, concomitant treatments and management of transplant related complications

Any clinically necessary treatment according to the investigator's decision and general or center specific guidelines and standards are permitted and should be followed if indicated.

5.1.4.1. Concomitant medication

At baseline and each subsequent visit until Day 100, patients will be asked what medications they are currently taking. Any medication that the patient receives or takes other than the study drug has to be recorded as concomitant medication, including herbal and other non-traditional remedies. Standard medication in the context of the haploidentical or MSD HSCT - defined for each center as applicable - and prophylactic medication as described below (see *section 5.1.4.2.*) will be documented with generic names, indication, start and stop dates and maximum dose with unit of measurement from Day 0 to Day 100. Study centers shall provide definitions for such standard medication. All other concomitant medications from baseline to Day 100 have to be documented in the eCRF with generic name, indication, route of administration, dose including unit of measurement and start and end dates (before study or date; ongoing or date).
During the follow-up phases, that is after Day 100 until the end of the study concomitant treatment with other cellular products (erythrocyte, thrombocyte or antigen-specific T cell infusions) will be documented, only. Additionally, medication at the time of development of an adverse event or serious adverse event will be documented in detail on the AE/SAE form.

5.1.4.2. Prophylaxis

5.1.4.2.1. Anti-allergic prophylaxis

Patients receiving ATG Neovii during conditioning should receive anti-allergic medication consisting of

- Dimetindene (0,1mg/kg BW, max. 4 mg/dose IV)
- Prednisolone (2 × 2 mg/kg BW)

on Days –10 to –8 and days -3 to -1, respectively, depending on the type of donor (see table 1 and 2). The medications can be replaced by any equivalent form of anti-allergic prophylaxis according to institutional guidelines and SOPs.

5.1.4.2.2. Prophylaxis of GVHD and rejection

Post-transplant immunosuppression is critical for the primary outcome parameter (GVHD and DFS/rejection and should be followed carefully!

The primary parameter for stable engraftment is a weekly assessment of the total chimerism until 100% donor chimerism is achieved.

For this purpose all patients will receive immunosuppressive prophylaxis consisting of:

- Mycophenolate mofetil (MMF) 4 x 300mg/m^2/d IV and
- Tacrolimus 0,03 mg/kg BW as a continuous infusion over 20h, adjusted to reach a serum level of 5ng/ml to 8ng/ml. Optimally the target serum level should be reached around Day 0.

In MSD the predominant intention of this treatment is prevention of GVHD whereas in haploidentically transplanted patients, immunosuppression is predominantly prevention of graft rejection.

The duration of immunosuppression differs between MSD and haplo-HSCT.

1. **Duration of immunosuppression:**
   a. **In MSD HSCT:**
   - MMF from day -1 until **day 180** post-transplantation
   - Tacrolimus from day -3 until **day 180** post-transplantation

   b. **In haploidentical HSCT:**
   - MMF from day -1 until **day 240** post-transplantation
   - Tacrolimus from day -3 until **day 240** post-transplantation

2. **Prerequisite: Weaning of immunosuppression** can be started thereafter with weekly monitoring of chimerism if:
   a. **the total chimerism remained stable at >90% throughout the preceeding post-HSCT period with**
   b. **no clinical signs of GVHD**
   c. **Exception**: If the patient develops **raising viral** titers before day 180 and 240, respectively, a careful reduction of immunosuppression with weekly monitoring of total chimerism can be tried in order to support antiviral therapy.
   Please contact your national coordinator or the lead investigator for further advice.

3. **Weaning of immunosuppression:**
   a. With fulfillment of any of the above criteria weaning of immunosuppression can be initiated with a weekly reduction of one of the immunosuppressive drugs (preferably MMF) by **10% per week for 2 consecutive weeks** under careful weekly monitoring of total chimerism.
   b. If the total chimerism remains stable **>90%** and **no signs of GVHD** were observed further weaning by a weekly reduction of **20%** can be tried. Again, with careful weekly monitoring of total chimerism until complete omission.
   c. Consecutively, the second immunosuppressive drug can be weaned according to the same schedule.

5.1.4.2.3. Prophylaxis of viral, bacterial and fungal infections

Patients will be treated prophylactically starting with the first day of the conditioning as described below.

The suggested prophylactic regimens can be replaced by any equivalent form of anti-infective prophylaxis according to institutional guidelines and SOPs.

Adult Patients

Fungal prophylaxis* (until CD4+ engraftment of 100 – 200 cells/µl is achieved):

- Ambisome 1 mg/kg/d twice a week starting with the conditioning
- Caspofungin 50mg/d starting with the conditioning three times per week
- Posaconazol Suspension 3 × 200 mg/d PO, tablet and IV 2 x 300mg on day 1, followed by 1 x 300mg
- Voriconazol 2 × 200 mg/d PO (1-0-1)

Attention with raising tacrolimus titers when using azoles in particular with regard to neurotoxicity!

Viral prophylaxis (until CD4+ engraftment of 100 – 200 cells/µl is achieved):

- Acyclovir 2 x 800mg/d PO or 30mg/kg/d IV

Antiviral prophylaxis with Letermovir is highly recommended, particularly in the haploidentical arm.

- Letermovir 480mg IV/PO once daily in case of a CMV donor-recipient mismatch

	Donor	Recipient	Letermovir Prophylaxis
CMV Status	+	+	+
	+	−	+
	−	+	+
	−	−	−

Pneumocystis jirovecii prophylaxis:

- Trimethoprim 160 mg + sulfamethoxazol 800 mg (Cotrim forte®), 3 × 1 tbl/week (1-0-0) or pentamidine inhalation 2- 3 times weekly starting with engraftment

The prophylaxis with acyclovir, pyrethamine and trimethoprim should be continued for the first year after transplantation. The dose of acyclovir can be reduced to 200 mg BID after neutrophil recovery. Furthermore, prophylaxis against infectious diseases should be adjusted to the clinical situation, and to drug toxicities, the occurrence of GVHD and the necessity of immunosuppressive treatment.

Pediatric Patients

Fungal prophylaxis* (until CD4+ engraftment of 100 – 200 cells/µl is achieved):

- Ambisome 1 mg/kg/d twice a week starting with the conditioning
- Caspofungin 50 mg/m^2/d starting with the conditioning
- Micafungin 1-3 mg/kg/d (max. 50mg) starting with the conditioning
- Posaconazole
  Note: verify correct dosage form; the delayed release tablet and oral suspension are not interchangeable!
  - **Suspension:**
    >13yrs: Suspension 3 × 200 mg/d PO, IV 2 x 300mg on day 1, followed by 1 x 300mg
    >8 months to 12yrs: 3 x 4mg/kg/d
  - **Delayed release tablet:**
    - **15-21 kg:** 1 x 100 mg
    - **22-30 kg:** 1 x 150 mg
    - **31-35 kg:** 1 x 200 mg
    - **36-40 kg:** 1 x 250 mg
    - **> 40 kg and adults:** 1 x 300 mg
- Voriconazol
  - **<12yrs:** 2 x 8mg/kg/d IV, 2 x 9mg/kg/d max. 350mg
  - **>12yrs and <50kg BW:** 2 x 8mg/kg/d IV **or** 2 x 9mg/kg/d PO max. 350mg
  - **>12yrs and >50kg BW:** 8mg/kg/d IV or 2 x 200mg PO

*Any of the suggested medications or according to institutional guidelines

Attention with raising tacrolimus titers when using azoles in particular with regard to neurotoxicity!

Viral prophylaxis (until CD4+ engraftment of 100 – 200 cells/µl is achieved):

- Acyclovir 30mg/kg/d IV followed by 40mg/kg/d PO
- Letermovir: in case of a CMV donor-recipient mismatch (see table above)
  - **12 – 18 yrs:** (no weight adjustment): 480mg 1x day oral or IV
  - **2-12 yrs:** 1x day

>30 kg:	Oral: 480 mg	IV: 240 mg
18-30 kg:	Oral: 240mg	IV: 120 mg
11-<18 kg:	Oral: 120 mg	IV: 60 mg

Pneumocystis jirovecii prophylaxis:

- Trimethoprim 160 mg + sulfamethoxazol 800 mg at a dosage of 5mg/kg Trimethoprim/day divided in 2 doses, 2 - 3 x weekly starting with engraftment IV/PO or pentamidine inhalation every 3 weeks

Penicillin G 25,000 U/kg BW/d starting with discharge for 2 years or according to institutional guidelines.

Polyclonal immunoglobulin 200 to 400 mg/kg BW IV according to institutional guideliens.

Furthermore, prophylaxis against infectious diseases should be adjusted to the clinical situation and to drug toxicities, the occurrence of GVHD and the necessity of immunosuppressive treatment.

5.1.4.2.4. Seizure prophylaxis

Adults:
Levetiracetam 2 x 500mg daily IV/PO starting day -10 prior to conditioning until Day +30 or discharge from inpatient care.

Children: Levetiracetam 2 x 10mg/kg daily IV/PO (max. 2 x 500mg daily) starting day -10 prior to conditioning until Day +30 or discharge from inpatient care.

5.1.4.3. Supportive care

Institutional standards for general supportive care after transplantation should be maintained and should include antimicrobial agents (see above), nutritional support and blood product support as necessary, if not otherwise indicated previously.
Note: Nutritional support and supplements as well as fluid replacement/volume substitution solutions given according to institutional guidelines will not be documented as concomitant medication.

Venous Access:
Patients will have an appropriate central venous access placed, as detailed by institutional standard practice, prior to beginning the conditioning regimen. A preferentially three-line, or alternatively two-line device (e.g. Hickman catheter) should be inserted. Documentation of the correct position of the device before start of conditioning by echocardiography or by X-ray is recommended.

The date of central line placement should be latest 1-2 weeks before start of conditioning. Wherever fertility preservation procedures need to be combined, a sufficient wound healing of 7-10 days before start of conditioning treatment needs to be considered.

Blood Products:

All blood products, except the infused TCR$\alpha\beta$ and CD19 cells depleted PBSC, will be irradiated in accordance with institutional standards. Recipients who are CMV negative should receive either CMV negative blood products (preferred) or leukocyte depleted blood products with study entry.

Nutrition:

A low microbial diet will be maintained while the recipient is in isolation. Parenteral nutrition should be initiated depending on the patient's needs and/or institutional standards.

Isolation:

Recipients will be maintained in single occupancy rooms with protective isolation per institutional guidelines.

5.1.4.4. Management of transplant-related complications

5.1.4.4.1. Transfusions

Leukocyte-poor platelets should be administered when the platelet count is **<20 × 10^9/L** or per institutional guidelines. If the platelets are repetedly refractory as documented by lacking platelet count rise within 24h, **transfusion refractory thrombocytopenia** needs to be considered when an appropriate PLT count rise at 1h post-transfusion was excluded.

In case of repetitive (on more than 3 consecutive days) non-response to adequately transfused platelet products and exclusion of alloimmunization, VOD/SOS must be taken into consideration according to the pediatric EBMT criteria [79]. Platelets must be transfused upon any active bleeding.

Packed red blood cell transfusions are recommended when hemoglobin is **<7 mg/dL** or as clinically indicated.

5.1.4.4.2. Febrile neutropenia

Broad-spectrum antibiotics should be administered intravenously according to institutional guidelines as first line treatment, adjusted according to antibiogram if blood cutures are positive.

5.1.4.4.3. Bacterial infections

Antibiotic treatments will be administered according to institutional guidelines.

5.1.4.4.4. Suspected / Confirmed viral reactivation

If CMV-, EBV-, ADV, HHV6- and BK virus reactivation is suspected because of raising PCR titers, specific preemptive antiviral therapy is strongly recommended:

- **ADV reactivation (blood):** cidofovir 5 mg/kg BW weekly or 2,5mg/kg 2x/week (lower renal toxicity) for 2 weeks followed by every 2 weeks or 3mg/kg BW once weekly. Use with adequate hydratation (2-3l/m^2/day) and

  Probenecid: Oral: 25 to 40 mg/kg/dose (maximum dose: 2,000 mg) administered 3 hours before cidofovir infusion and 10 to 20 mg/kg/dose (maximum dose: 1,000 mg) at 2 to 3 hours and 8 to 9 hours after cidofovir infusion or 1000 mg or 1,250 mg/m^2/dose administered 3 hours prior to cidofovir, followed by 500 to 1,250 mg/m^2/dose 1 to 2 hours and 8 hours after completion. In case of increasing ADV copy numbers in blood consider ADV specific T cells.
- **CMV reactivation**: start preemptive empirical therapy with therapeutic doses of ganciclovir or foscavir immediately after the first positive PCR. If CMV DNA will be detected in urine or throat prior to transplant, eradication with ganciclovir or foscavir should be tried before start of the conditioning regimen. In case of treatment refractory increasing copy numbers, switch to the complementary drug (ganciclovir or foscavir), request CMV drug resistance testing.
- **HHV6 reactivation:** HHV-6B reactivation is reported to occur in 30% to 70% of patients undergoing allogeneic HCT, typically between 2 and 4 weeks after HSCT. Encephalitis is the most clearly established clinical manifestation of HHV-6B reactivation in allogeneic HSCT recipients and, while it may result in substantial morbidity, it occurs in a small subset of these patients. HHV-6B is also suspected of causing bone marrow suppression, triggering GVHD, CMV reactivation, pneumonitis, and raising TRM. No antiviral agent has been approved for the treatment of HHV-6B infection. However, in vitro studies demonstrate that **foscarnet**, **ganciclovir** and **cidofovir** have antiviral activity against HHV-6B.
  The possibility of chromosomally integrated or inherited HHV-6 should be considered in the setting of extremely high and persistent levels of HHV-6 DNA in the peripheral blood, particularly if the patient has no clinical signs of HHV-6.
  Note of caution: It might be worthwhile to biopsy a skin rash in patients with newly rising HHV6 titers to confirm the most plausible clinical diagnosis of aGVHD before starting steroids.

- **BK Polyomavirus (BKPyV/BK):** BK polyomavirus (BK) is a common cause of hemorrhagic cystitis during the early post-engraftment period which occurs most commonly at 3 to 6weeks post-HSCT. BKPyV persistently infects renal tubular epithelial cells and also replicates in urothelial cells. Risk factors for BKPyV-associated hemorrhagic cystitis include a myeloablative conditioning regimen in the setting of human leukocyte antigen (HLA) mismatch, particularly with haploidentical or umbilical cord blood grafts. Those patients who had a positive urine BKPyV polymerase chain reaction (PCR) pre-transplant had a significantly higher risk of developing hemorrhagic cystitis (58%). BKPyV plasma titers have a relevant predictive value. Detection of BKPyV DNA in plasma appears to be a significant marker of BKPyV-associated hemorrhagic cystitis in HCT recipients. Levels of BK viremia >10,000 copies/mL are thought to predict renal and urologic outcomes in HCT recipients. Meticulous viral monitoring is therefore pivotal in order to install preemptive treatment either locally via intravesical catheter instillation of cidofovir [80-83] or IV cidofovir similar to ADV treatment (see above) or according to institutional guidelines.
- **PTLD**: in case of increasing EBV copy numbers or >1000 copies/µL rituximab 375 mg/m2 once weekly for 4 weeks.

If viral titers are rising continuously despite pre-emptive antiviral therapy, consider treatment with (donor derived or third party) virus-specific T cells early!

5.1.4.4.5. Graft failure

Primary or secondary GF, defined as follows, will be considered a treatment failure.

Primary graft failure:
ANC <0.5 × 10^9/L by Day 28 and platelets <20 × 10^9/L
(Hemoglobin <8 g/dL Is omitted due to inclusion of donors with SCD heterozygosity)

Secondary graft failure: ANC <0.5 × 10^9/L after initial engraftment not related to infection, or drug toxicity, unresponsive to growth factor therapy and/or other intervention until 12 months post-HSCT.

Patients will be treated according to institutional guidelines at the investigator's discretion. Mixed chimerism should not be considered GF a priori (see below).
In secondary GF a transplant-associated macrophage activation syndrome (TAMAS) should be considered, in particular in haploidenticall transplanted SCD patients with rising or poorly controlled viral titers or other immunological triggers in patients under immunosuppression.
Management of GF and/or TAMAS should be discussed with the national coordinating investigator or the respective leading investigator.

5.1.4.4.6. Transplant-associated macrophage activation syndrome (TAMAS)

Macrophage activation syndrome (MAS) is a serious and historically rare life-threatening complication now increasingly observed in recipients of allogeneic HSCT. Excessive uncontrolled and dysregulated immune activation with proliferation of T lymphocytes and macrophages leads to hyperinflammatory responses with hypercytokinemia and hemophagocytosis. Similar but different from other primary or secondary hemophagocytic lymphohistiocytosis (HLH) there is no suitable definition for transplant associated MAS (TAMAS). Available HLH criteria do not reflect the specificity of MAS presenting in patients following allogeneic HSCT and therefore should be used cautiously. TAMAS is usually diagnosed after engraftment or due to primary engraftment failure in median 1 month after HSCT, rarely even several months after. Not every time it was diagnosed in patients with GvHD or viral reactivation, although GvHD or viremia may trigger in some patients its development. The outcome of patients who developed TAMAS is inferior to general results. Often patients suffer from non-engraftment or graft failure and need second transplantation. TAMAS is an adverse event of specific interest (see below).

Significant clinical and laboratory:
- Primary or secondary cytopenia responsive to transfusions (not transfusion refractory)
- Rising ferritin or absolute ferritin above 5000 ng/mL

- Persistent fever
- Triglycerides above 265 mg/dL

Rare and less sensitive:

- Marrow hemophagocytosis
- Max sCD25 above 2400 UI/mL

Less specific:

- Splenomegaly
- Fibrinogen below 150 mg/dL
- Any neurological involvement

Therapy should be tailored based on local experience and drugs used for primary or secondary HLH. To specifically target TAMAS we do recommend measuring cytokines to justify use of targeted therapy (etanercept, emapalumab, tocilizumab, etc.). Steroids were used mostly with limited efficacy in some patients.

5.1.4.4.7. Mixed chimerism

This paragraph is valid predominantly with hapoloidentical transplant patients but can be applied also in MSD HSCT.
Measurement of weekly peripheral blood total chimerism after engraftment is pivotal in particular in T cell depleted haploidentically transplanted patients since rejection and GF are the major transplant-related morbidity in patients with SCD and a high pre-transplant transfusion frequency.
Hyperacute rejection was not observed in the pilot group of 25 patients treated with the current conditioning and post-transplant immunosuppressive regimen. If the post-transplant immunesupression is executed meticulously including adequate tacrolimus serum level requirements, hyperacute rejection is rarely to be expected.

The exclusion of DSA positive as well as major AB0 incompatible donors is pivotal for the experimental arm. (Exclusion criteria!).

Management of mixed chimerism:

Split chimerism analyses are indicated when institutional total chimerism analyses dropped below 90%!

The trial offers central PCR based split chimerism analysis. In order to properly perform split chimerism analyses, a **10ml PB EDTA** sample **before** start of transplant from **donor** and **recipient** has to shipped to Regensburg to the address indicated below.

Chimerism analysis by PCR is being performed using the Mentype Chimera kit employing a multiplex reaction with 12 highly polymorphic STS markers and a gender specific marker (www.biotype.de). From each patient of all participating centers genomic DNA from peripheral blood (PB) and bone marrow (BM) will be isolated manually (Qiagen-kit) at the institute of clinical chemistry and laboratory medicine in Regensburg (Prof. Aslanidis) and an initial chimerism-PCR performed upon entry to the study.
For monitoring of split chimerism sample material (PB, BM) should be sent to the address indicated below:

Petra Turowski
Head Technician
Clinical Trials Centre of the
Department of Pediatric Hematology and Oncology (SPOH)
University Childrens Hospital
Regensburg
Room Nr C5 3.502
Franz-Josef-Strauß-Allee 11
93053 Regensburg

Please contact Ms Turowski via email prior to shipment at:

SCD.SPOH@ukr.de

or

Tel.: +49(0)941 944 12066

Fax: +49(0)941 944 2067

After cell sorting (PB (CD3+, CD14+, CD56+), BM (CD3+, CD14+, CD235a+), genomic DNA will be isolated from each sorted material and analyzed by chimerism-PCR.

There are 3 situations where a slowly developing mixed chimerism can emerge:

a. **During weaning of the immunosuppression**: **Raise** the immunsupression to the last dose of a full donor chimerism and delay weaning by 4 weeks. Continue weekly total chimerism level measurements. Send a blood sample to Regensburg for split chimerism analysis. (Shipping information in *appendix B.13*)
b. **Off immunosuppression:** In patients who develop a mixed chimerism late after omission of immunosuppression, send a blood sample to the central lab for split chimerism anaylsis in order to assess the status of the graft. No further action is required until split chimerism analyses are available. (Shipping information in *appendix B.13*)
c. **During viral reactivation/infection:** Pivotal is to control viral reactivation as quickly as possible. Discuss careful reduction of immunosuppression with national PI or lead PI.

In cases a. and b. the split chimerism analysis will help you in your decision process. The expected situation is a **dissociated split chimerism** in which the myeloid chimerism (peripheral blood monocyte count) is predominantly donor and the T cell (CD3) chimerism can be predominantly recipient.
In patients off immunosuppression with a **dissociated split chimerism no action** is required. Continue total chimerism assessment monthly for 2 months or until full chimerism is achieved. If mixed chimerism persists, re-submit a sample for a follow-up split chimerism analysis.
In most instances the donor CD3 split chimerism will raise continuously. This is usually not prominent in the total chimerism analysis but confirms the maturation of the T depleted graft. Continue with monthly chimerism analysis.
In both cases, chimerism data can be corroborated with hemoglobin electrophoresis where in parental T cell depleted haploidentical HSCT, a HbS of 30-40% can be expected and reflects a full donor chimerism (in haploidentical donors with a carrier status).
In case of a mixed chimerism throughout all hematopoietic cell lines measured, contact the trial office for advice on further management.

5.1.4.4.8. Neurotoxicity

The incidence of neurotoxicity (NT) is a frequent and potentially life-threatening complication. Almost two thirds of the neurologic episodes (63%) occur within 100 days after transplantation [63]. The non-specific clinical manifestations and multiplicity of radiological patterns raise diagnostic challenges.

Due to the elevated risk for NT in SCD patients, a prophylactic treatment for all included patients is scheduled in the protocol from the start of conditioning with levetiracetam.

To minimize the risk of calcineurin-inhibitor (CNI) associated NT, immunosuppression is performed as first-line-therapy with tacrolimus with a starting dose of 0.03 mg/kg given as a 20-h-infusion with a dose adjustment to target a serum level of 7 ng/ml (range 5-8 ng/ml). Beware, CNI-related NT may occur even in situations of correct serum levels.

Close blood pressure monitoring and aggressive treatment of hypertension according to institutional guidelines is recommended.

Diagnosis of NT:

Consider NT in case of neurological symptomas such as:

- Recurrent Headache
- Sleep disorder
  - Sleep disorder

  - Insomnia
  - Nightmares
- Encephalopathy:
  - Encephalopathy
  - Cognitive disorder
  - Confusional state
  - Depressed level of consciousness
  - Disturbed attention
  - Hypersomnia
  - Leukoencephalopathy
  - Memory impairment
  - Mental status changes
  - Paranoia
  - Somnolence
  - Stupor
  - Dyscalculia
- Delirium:
  - Agitation
  - Delirium
  - Delusion
  - Disorientation
  - Hallucination
  - Hyperactivity
  - Irritability
  - Restlessness
- Ataxia
- Visual disturbance
- Tremor, muscle spasms, muscular weakness
- Seizures
- Cortical blindness
- Aphasia
- Ataxia
- Papillary edema

For quantification of SCD related post-HSCT NT we will use the 'ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells'. The CART related NTs are unique toxicities related to cytokine release and called therefore 'cytokine release syndrome' (CRS) and neurologic toxicity. The assessment and grading of these toxicities varied considerably across clinical trials and across institutions, making it difficult to compare the safety of different products and hindering the ability to develop optimal strategies for management of these toxicities.
The observed clinical phenotype resembles much the SCD related NT with most probably the same background with regard to a vascular pathophysiology so that the presented grading system for CART related CRS and NT will be used in this trail [84].

Encephalopathy Assessment Tools for Grading of ICANS (*appendix B.7b*)

CARTOX-10 [12]	ICE
• **Orientation:** orientation to year, month, city, hospital, president/prime minister of country of residence: 5 points • **Naming:** ability to name 3 objects (eg, point to clock, pen, button): 3 points • **Writing:** ability to write a standard sentence (eg, "Our national bird is the bald eagle"): 1 point • **Attention:** ability to count backwards from 100 by 10: 1 point	• **Orientation:** orientation to year, month, city, hospital: 4 points • **Naming:** ability to name 3 objects (eg, point to clock, pen, button): 3 points • **Following commands:** ability to follow simple commands (eg, "Show me 2 fingers" or "Close your eyes and stick out your tongue"): 1 point • **Writing:** ability to write a standard sentence (eg, "Our national bird is the bald eagle"): 1 point • **Attention:** ability to count backwards from 100 by 10: 1 point

CARTOX-10 (left column) has been updated to the ICE tool (right column). ICE adds a command-following assessment in place of 1 of the CARTOX-10 orientation questions. The scoring system remains the same.
Scoring: 10, no impairment;
7-9, grade 1 ICANS;
3-6, grade 2 ICANS;
0-2, grade 3 ICANS;
0 due to patient unarousable and unable to perform ICE assessment, grade 4 ICANS

ASTCT ICANS Consensus Grading for Adults (*appendix B.7a*):

ASTCT ICANS Consensus Grading for Adults

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
ICE score*	7-9	3-6	0-2	0 (patient is unarousable and unable to perform ICE)
Depressed level of consciousness†	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse. Stupor or coma
Seizure	N/A	N/A	Any clinical seizure focal or generalized that resolves rapidly or nonconvulsive seizures on EEG that resolve with intervention	Life-threatening prolonged seizure (>5 min); or Repetitive clinical or electrical seizures without return to baseline in between
Motor findings‡	N/A	N/A	N/A	Deep focal motor weakness such as hemiparesis or paraparesis
Elevated ICP/ cerebral edema	N/A	N/A	Focal/local edema on neuroimaging§	Diffuse cerebral edema on neuroimaging; decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing's triad

ICANS grade is determined by the most severe event (ICE score, level of consciousness, seizure, motor findings, raised ICP/cerebral edema) not attributable to any other cause; for example, a patient with an ICE score of 3 who has a generalized seizure is classified as grade 3 ICANS.
N/A indicates not applicable.

** A patient with an ICE score of 0 may be classified as grade 3 ICANS if awake with global aphasia, but a patient with an ICE score of 0 may be classified as grade 4 ICANS if unarousable.*
† Depressed level of consciousness should be attributable to no other cause (eg, no sedating medication).
‡ Tremors and myoclonus associated with immune effector cell therapies may be graded according to CTCAE v5.0, but they do not influence ICANS grading.
§ Intracranial hemorrhage with or without associated edema is not considered a neurotoxicity feature and is excluded from ICANS grading. It may be graded according to CTCAE v5.0.

ASTCT ICANS Consensus Grading for Children (*appendix B.7c*):

ASTCT ICANS Consensus Grading for Children

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
ICE score for children age ≥12 years*	7-9	3-6	0-2	0 (patient is unarousable and unable to perform ICE)
CAPD score for children age <12 years	1-8	1-8	≥9	Unable to perform CAPD
Depressed level of consciousness†	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Unarousable or requires vigorous or repetitive tactile stimuli to arouse; stupor or coma
Seizure (any age)	N/A	N/A	Any clinical seizure focal or generalized that resolves rapidly or nonconvulsive seizures on EEG that resolve with intervention	Life-threatening prolonged seizure (>5 min); or Repetitive clinical or electrical seizures without return to baseline in between
Motor weakness (any age)‡	N/A	N/A	N/A	Deep focal motor weakness, such as hemiparesis or paraparesis
Elevated ICP/ cerebral edema (any age)	N/A	N/A	Focal/local edema on neuroimaging§	Decerebrate or decorticate posturing, cranial nerve VI palsy, papilledema, Cushing's triad, or signs of diffuse cerebral edema on neuroimaging

CANS grade is determined by the most severe event (ICE or CAPD score, level of consciousness, seizure, motor findings, raised ICP/cerebral edema) not attributable to any other cause. Baseline CAPD score should be considered before attributing to ICANS.

** A patient with an ICE score of 0 may be classified as grade 3 ICANS if awake with global aphasia, but a patient with an ICE score of 0 may be classified as grade 4 ICANS if unarousable.*

† Depressed level of consciousness should be attributable to no other cause (eg, no sedating medication).
‡Tremors and myoclonus associated with immune effector cell therapies may be graded according to CTCAE v5.0, but they do not influence ICANS grading.
§ Intracranial hemorrhage with or without associated edema is not considered a neurotoxicity feature and is excluded from ICANS grading. It may be graded according to CTCAE v5.0.

For cognitive deficits/encephalopathy in children <12 years, please use the ICE/CAPD Score (*appendix B.7d*):

Encephalopathy Assessment for Children Age <12 Years Using the CAPD

Answer the following based on interactions with the child over the course of the shift					
	Never, 4	Rarely, 3	Sometimes, 2	Often, 1	Always, 0
1. Does the child make eye contact with the caregiver?					
2. Are the child's actions purposeful?					
3. Is the child aware of his/her surroundings?					
4. Does the child communicate needs and wants?					
	Never, 0	Rarely, 1	Sometimes, 2	Often, 3	Always, 4
5. Is the child restless?					
6. Is the child inconsolable?					
7. Is the child underactive; very little movement while awake?					
8. Does it take the child a long time to respond to interactions?					

(Adapted from Traube et al [Traube C, Silver G, Kearney J, et al. Cornell Assessment of Pediatric Delir ium: a valid, rapid, observational tool for screening delirium in the PICU. Crit Care Med. 2014;42:656–663.)

For patients age 1-2 years, the following serve as guidelines to the corresponding questions:
1. Holds gaze, prefers primary parent, looks at speaker.
2. Reaches and manipulates objects, tries to change position, if mobile may try to get up.
3. Prefers primary parent, upset when separated from preferred caregivers. Comforted by familiar objects (ie, blanket or stuffed animal). 4. Uses single words or signs.
5. No sustained calm state.
6. Not soothed by usual comforting actions, eg, singing, holding, talking, and reading.
7. Little if any play, efforts to sit up, pull up, and if mobile crawl or walk around.
8. Not following simple directions. If verbal, not engaging in simple dialog with words or jargon

Imaging

Cerebral MRI is the key investigation for the diagnosis of PRES. MRI is superior to cerebral CT for the diagnosis of PRES. Although CT may be easier to obtain first, CT findings are often normal or nonspecific.

Basic MRI Sequences to be considerd for imaging are:

- FLAIR
- T2
- T2-GRE
- TOF-Angio

Toxicity-MR Sequences:

- Diffusion-weighted magnetic resonance (dwi) images
- Apparent diffusion coefficient (ADC) images
- T2
- T2-GRE
- TOF-Angio
- T1 with and without contrast media
- FLAIR

Management of NT

Primary management:

Dexamethasone 0,2mg/kg up to 3 x/day (in children), 8mg/dose up to 3 x/day (in adults).

1. Management of mild/moderate NT (ASTCT ICANS Grade 1 & 2)
   In case of the occurrence of clinically overt neurological signs and symptoms indicative for mild or moderate NT (*Grade 1 & 2*), correct dosage of tacrolimus within the target range of 5-8 ng/ml should be assured immediately and corrected where needed. Other potentially neurotoxic medications should be checked and replaced, where possible. If the serum level of tacrolimus is within the correct range, a watch-and-wait-strategy can be applied as mild symptoms have a high rate of spontaneous resolutions.
   In case of persistence or any worsening of symptoms, refer to management of severe NT.

2. Management of severe NT (ASTCT ICANS Grade 3 & 4)
   In case of the occurrence of clinically overt neurological signs and symptoms indicative for severe NT (Grade 3 & 4), clinical management of the acute situation should be performed according to institutional guidelines, like anticonvulsive treatment in case of seizures and transfer to the Intensive Care Unit where clinically indicated.
   As for mild/moderate NT, correct dosage of tacrolimus within the target range of 5-8 ng/ml should be assured immediately and corrected where needed.
   If the serum level of tacrolimus is within the correct range, and severe neurologic symptoms occur, tacrolimus should be discontinued, and immunosuppression should be switched to everolimus.

5.1.4.4.9. Sickle related pain management

Approximately 40% of SCD patients experience pain episodes at or around the time of stem cell engraftment, very similar to classical engraftment syndrome. This 'engraftment pain' after HSCT is similar in its experience of pain crises, well known to SCD patients. The cause is unknown. Classical explanations for the pathophysiology of the painful crises focused on occlusion of small blood vessels by irreversibly sickled erythrocytes and resulting ischemic pain. More recently, and especially in the development of non-crisis pain in patients with SCD possible explanation have been put forward to include neural and inflammatory mechanisms. These indicate roles for pathological erythrocyte adhesion, endothelial activation, endothelial damage, and neural mechanisms in non-crisis pain phenomena [85, 86]. Hypotheticall, the chronic and systemic damage of the endothelium in SCD patients leads to multiple osteonecrosis in advanced stage SCD patients. The pain episodes are substantial, requiring high-dose and prolonged pain therapy. Even opioids sometimes cannot resolve engraftment

pain satisfactorily. In case of suspected engraftment pain, the national coordinating investigator or the respective leading investigator can be consulted for advice regarding management of uncontrollable pain episodes.

Pain Assessment

To achieve an effective pain management a frequent reassessment of pain is required and management of opioid tolerance or opioid side effects. The subjective nature of pain may make an assessment of the severity of a patient's non-crisis pain difficult, but Guidelines for health professionals provided recommendations on evaluating an assessment of the severity of a patient's non-crisis pain. Depending on local standards, most commonly used instruments to assess pain are numeric scales, in which clinicians ask patients to report their pain level as a number from zero to 10. In case of children, the physicians use picture pain scales such as Oucher scale or Wong-Baler scale to assess pain intensity. The assessment should be done after each change in treatment regimen and when sufficient time has relapsed for the drug to reach optimal efficacy. Further, pain episodes, age, chronic comorbidities and laboratory values like oxygen saturation, blood pressure and temperature should be considered in patient's assessment. An FDA approved (DDT COA #000057) sickle cell pain diary will be also made available for the trial in several languages.

Non-pharmacologic Management

Depending on local standards, non-pharmacological treatment options should be used to achieve a reduction of pharmacological analgetics. Physical strategies such as adequate hydration, massages, blood transfusions and physiotherapy will be helpful in reducing analgetics.

Pharmacological Management

The foundation of the management of non-crisis sickle cell pain are opioid analgetics and typically a severe episode with pain scores >7 out of 10. In consequence the individual opioid dose varies depending on the severity of the pain, the clinical situation (infection, engraftment et al), age of the patient, the existing damage to the vessels or existing osteonecrosis, and the primary use of opioids (opioid tolerance). Normally these patients require much higher doses of opioids and combination pain relief strategies with codeine and ketamine than standard patients. This pain management paragraph describes our step-by-step approach of pain therapy in the case of severe non-crisis SCD pain under haploidentical stem cell transplantation. In any case institutional guidelines and SOPs have priority over these recommendations.

In case patients are on chronic pain management with opioids, the oral medication should be transferred with equianagesic dosing into a parenteral formulation ie as continuous infusion, best before start with any transplant-related procedure and latest before conditioning.

I. Pain score > 7, severe headache; starting doses for parenteral opioid analgetics without knowing the patient'manns opioid requirements:

Morphine 0.02 mg/kg/h c.i. (bolus doses iv 0.02 mg/ kg bw q2-3h)

or

Hydromorphone 0.005 mg/kg/h c.i. (bolus doses iv 0.004 mg/ kg bw q2-3h)

combine with

Naloxone 0.25 µg/kg/h c.i.(suppress side effects of opioids-itchy!)

combine with

Metamizole (Dipyrone):

Children: 60 mg/kg/24h c.i.

Adults: 1 - 2,5g up to 4 x /daily

combine with

Gabapentine (Neurontin) from 200 mg to 600 mg oral q8h (children)

or

Amitryptiline PO start with 10 mg at night with an individual increase of 10 mg (electrocardiogram recommended before application (Cave: long-QT-syndrom)
(as nontricyclic antidepressant *analgesics in adult patient*)

Titration of the required dosages of the opioid must be applied to each individual patient with the criteria of clinical response to the pain medication (dropping pain score).

In case of insufficient pain control combine with

Low dose dexmedetomidine (Dexdor, α_2-adrenoceptor agonist, clonidine agonist) dosage 0.2 up to 0.9 mg/kg/h as c.i.

In case of insufficient pain control, combine with

Low dose ketamine 25 mg up to 50 mg/24h as c.i.

Important: Recommended doses do not apply to patients with renal or hepatic insufficiency or other conditions affecting drug metabolism and kinetics. With higher dosages of dexmedetomidine or ketamine a transfer to the intensive care unit is highly recommended.

Generally, it is recommended, that pain specialists or pain clinic anesthesiologists should be involved early in the pain management of SCD patient.

5.1.4.4.10. Acute GVHD

The incidence of acute GVHD is one of the primary endpoints and needs to be monitored and reported meticulously.

Skin GVHD: The origin of exanthemas in particular early post-transplant are often difficult to differentiate and include allergic or medication related skin reactions, thiotepa related toxicity or infectious skin lesions, that have to be differentiated from acute skin GVHD (including HHV6, see above). Therefore, in case of an exanthema, that cannot clearly be related to any cause, a confirmatory skin biopsy with microbiological and histopathological analysis is strongly recommended and is pivotal in case of failure of first-line treatment. In addition, photo-documentation is highly recommended.

Gut GVHD: Diarrhea post-transplant should be handled similarly including exclusion of infectious causes. In case of doubt an endoscopic biopsy is highly recommended and mandatory in case of failure of first-line treatment.

Liver GVHD: In the presence of a significant rise in direct bilirubin, AP and ALT failing to respond to initial treatment, which cannot be clearly related to one cause by laboratory workup and in the presence of treatment relevant differential-diagnosis, a liver biopsy is recommended.

Grading of aGVHD:
Grading of aGVHD should be performed according to the MAGIC criteria [87] (*appendix B.1*) and the use of the EBMT GVHD App is recommended.

Management of aGVHD
Management of aGVHD should be performed according to institutional guidelines. The recommendation of the trial is a standardized approach for first-line treatment starting with prednisolone (or equivalent methylprednisolone dose) at 2 mg/kg IV or PO.
In case of progression after 3 days, no improvement of the clinical symptoms after 1 week of treatment or persistence of active symptoms after 2 weeks of treatment we recommend the start of 2nd line treatment either according to institutional guidelines or the following second line approach: extracorporal photopheresis (ECP) [88] starting with 3 times per week until control of aGVHD followed by ruxolitinib [89]. We do not recommend the combination of mTOR inhibitors and CNI due to increased risk for TMA in

SCD patients. With improvement of the clinical symptoms we intend to reduce steroids first due to the increased risks for steroid induced osteonecrosis in SCD patients.

5.1.4.4.11. Chronic GVHD

Chronic GVHD should be diagnosed and graded according to the 2014 NIH consensus guidelines [17] (*appendix B.2*). In case of diagnostic uncertainity, we recommend histopathological confirmation prior to any medical intervention. In case of failure of 1st line treatment in clinically obvious cases, biopsies should be performed and sent to Regensburg for confirmatory assessment. Screening and diagnosis of cGVHD includes regular lung function tests of asymptomatic patients.
Based on randomized trials the preferred 1st line treatment consists of prednisolone (or equivalent methylprednisolone dose) 1mg/kg/day given once daily which may be supplemented with CNI or in case of contraindication or increased mortality risks (progressive onset, lower GI symptoms, platelets < 100/nl) with an mTOR-inhibitor (no combination with CNI as outlined above), ECP or MMF. Optimal second line treatment has not yet been established. Therefore, patients with steroid-refractory cGVHD should be treated according to international and institutional guidelines. It should be considered that according to the recommendations of the consensus conference in clinical practice in cGVHD initial second line treatment should include agents with an adequate safety profile and well-documented activity, whereas agents with significant side effects should be reserved for third- or fourth-line treatment.

5.1.5. Health-related quality of life (HRQoL)

SCD adversely affects HRQoL significantly when compared to children without the disorder [90]. QoL assessment in SCD patients is strongly recommended by international guidelines [91]. HRQoL is generally conceptualized as a multidimensional construct referring to patients' and parents' perceptions of the impact of disease and treatment by HSCT on their physical, psychological, social and school functioning and well-being as defined by the WHO. We anticipate that patients in addition to the alleviation of clinical manifestations of SCD will demonstrate a significant decline in mean symptom intensity and an improvement in most aspects of HRQoL by one year after HSCT. While our endpoint is the change between the baseline and one-year assessments, we will also examine HRQoL at day +100/ +180 and at 2 years after HSCT in order to better understand the trajectory of changes during the follow-up period. Although self-reporting is considered the best method to measure HRQoL, in those too ill or too young to report their HRQoL, a caregiver as proxy is acceptable.

<u>HRQoL Evaluation:</u>

The PedsQl 4.0 23-item generic core scales encompass physical functioning (8 items), emotional functioning (5 items), social functioning (5 items) and school functioning (5 items). The PedsQL generic core scales have demonstrated reliability, validity and responsiveness in SCD [92-95]. To create the total PedsQL score, the mean is computed as the sum of all items over the number of items answered on all scales. Each item is rated on a 5-point Likert scale. Raw scores are transformed into standardized scores on a scale of 0-100, with higher scores representing higher levels of functioning. The reliability, internal consistency, and validity of the PedsQL questionnaire have been assessed in pediatric patients with various acute and chronic disorders and in healthy pediatric cohorts [96, 97]. The PedsQL SCD module scales evidenced excellent feasibility and reliability for the total scale scores [98]. The 43-item PedsQL SCD module encompasses nine scales: pain and hurt (9 items), pain impact (10 items), pain management (2 items), worry I (5 items), worry II (2 items), emotions (2 items), treatment (7 items), communication I (3 items), communication II (3 items). The format instructions, likert response scale and the scoring method are identical to the PedsQL 4.0 generic core scales, with higher scores indicating better HRQoL and lower SCD symptoms.

The PedsQL Stem Cell Transplant (SCT) module covers SCT- and cGVHD-related problems and consists of the domains: pain and hurt, fatigue/sleeping problems/weakness, nausea, worry/anxiety about disease/treatment, nutritional and neurocognitive problems, communication about disease/treatment, loneliness, physical functioning and additional somatic complaints (pruritus, skin inflammation, oral problems, eyes or breathing).

Both the generic PedsQL and the SCT-specific scales showed high internal consistency, with Cronbach alpha levels of ⩾0.70 in almost all scales. Most problems were detected within the HRQoL domains of physical functioning and pain. The summary scores of the generic PedsQL and the PedsQL Stem Cell Transplant module showed high correlations and discriminated between patients with and without cGVHD in a pilot study[99].

Measures:

HRQoL will be assessed using the

- PedsQL 4.0 generic core scales,
- PedsQL SCD module
- PedsQL SCT module (Lawitschka et al, BMT 2014)
- Child self-report version and parent-proxy
- German, english language

Timepoints

- Baseline: before HSCT, ideally before treatment with hydroxyurea
- Day 100, 180, 1 year and 2 years after HSCT
- administered on a single day, +/- 7 days

For adults the standardized EQ-5D and FACT-BMT Quality of Life (QoL) questionnaires at the identical timepoints will be used.

All Qol questionnaires will be available either paper based or online for assessment during follow-up outpatient visits on site.

5.1.6. Enrolment in other clinical trials

All patients undergoing allogeneic HSCT are at risk of the same complications, namely GVHD, infection and GF. These complications may require additional experimental treatment after infusion of the IMP. Patients with these complications are often not suitable for standard therapy or unlikely to benefit from, therefore enrolment in appropriate clinical studies might be required according to the investigator's judgment. Thus, inclusion in another clinical trial after the primary endpoint of this study is reached, will be allowed for indications described in the following. Enrollment in any other clinical study is not allowed according to this study protocol. Patients, who will be included in another study protocol before the primary endpoint is reached, will be excluded from the 'per protocol analysis'.

Treatment of Infections

Infections - bacterial, viral, fungal and parasitic - continue to be a substantial cause of death after allogeneic HSCT. Novel approaches are still required since standard therapies have not been established to date. Accordingly, patients suffering from infections and failing standard therapy will be allowed to be included in appropriate clinical trials provided the new study does not interfere with the endpoints of the present study according to the investigator's judgment. Treatment of infections and selection of an appropriate clinical study will follow local guidelines of the study center.

5.2. Study assessments

5.2.1. Quality assessment of the graft

The cell content of PBSC grafts (the individual IMPs) after TCR$\alpha\beta$ and CD19 depletion will be analysed to ensure the quality of the graft for transplantation and to evaluate the performance of the Miltenyi CliniMACS® or the Prodigy® TCR$\alpha\beta$/CD19 systems.

The following parameters will be assessed:

- The percentage of recovered viable $CD34^+CD45^+$ cells after TCR$\alpha\beta$ and CD19 depletion procedure:

target value ≥95%.

- Log Depletion of TCR$\alpha\beta$ cells
- Log Depletion of $CD19^+$ cells
- Cell counts: $CD34^+CD45^+$ HSC, $CD20^+$ B cells, $CD56^+CD16^+$ NK cells, TCR$\alpha\beta$ and TCR$\gamma\delta$ cells, $CD3^+$ cells and $CD45^+$/WBC cells analysed by flow cytometry after processing prior to transplantation
- The percentage of recovered viable $CD45^+$ cells after TCR$\alpha\beta$ and CD19 depletion procedure: target value ≥90%
- Hematocrit value in the graft in mL/mL erythrocytes
- Number of grafts containing ≥10 × 10^6 $CD34^+CD45^+$cells/kg BW of the recipient
- Number of grafts containing ≤5 × 10^4 TCR$\alpha\beta$ cells/kg BW of the recipient
- Number of grafts containing ≤1 × 10^5 $CD20^+$ cells/kg BW of therecipient.

Additional parameters for IMP specification documented in the 'certificate of analysis' (see *section 4.4.1*) are

- Visual control (bags undamaged, no cell aggregates visible) and sterility (assessed but not relevant for IMP release).

5.2.2. Donor baseline evaluation

The donor will have been assessed regarding medical eligibility for stem cell donation prior to and independent of all study procedures according to guidelines and standards for stem cell donation. This has to be done at the individual collection center. Furthermore, donors will have given their informed consent for stem cell donation and all procedures related to it prior to and independent of the inclusion of the respective patient (recipient) in this present study. Donors will be allocated an individual donor ID by the collection center according to the following algorhythm.

Some of the data documented at the collection center during evaluation for stem cell donation will be transferred to the recipient's eCRF. Data transferred will comprise

- Donor ID
- Demographic characteristics
    - Height in cm
    - Weight in kg
    - Date of birth
    - Ethnic origin
    - Gender
    - Consanguinity
    - Family history
    - Social history
- Results of routine laboratory tests (see below for details)
- Prior to the inclusion of the respective patient the donor will be asked to visit the transplantation center for one baseline visit. During this visit he/she will have to give his/her study related written informed consent and study specific blood samples will be collected (see below). No study specific procedures including transfer of previously collected data will be performed without written informed consent of the donor. After obtaining written informed consent and confirmation of in-and exclusion critieria, samples of peripheral blood will be collected as specified (see *section 5.2.2.1*.) Samples will be processed and stored under appropriate conditions until shipment for subsequent analysis.

5.2.2.1. Donor baseline laboratory panel

The following data have to be entered in the eCRF for baseline evaluation of the donor, as possible:

Hematology

- Hemoglobin
- Hematocrit
- Erythrocytes
- Leukocytes
- Thrombocytes
- Reticulocytes
- MCV and MCH
- Differential Blood count

Clinical chemistry

Electrolytes

- Sodium
- Chloride
- Potassium
- Magnesium
- Calcium
- Phosphate

Substrate

- Glucose
- Total bilirubin
- Conjugated bilirubin
- Unconjugated bilirubin
- Creatinine
- Urea (BUN)
- C-reactive protein (CRP)
- Total protein
- Albumine
- Uric acid

Enzymes

- Alanine-aminotransferase (ALAT)
- Aspartate-aminotransferase (ASAT)
- Gamma-glutamyl transferase (GGT)
- Lactate dehydrogenase (LDH)

Coagulation

- INR
- Fibrinogen
- Prothrombin time (PT, Quick)
- Partial thromboplastin time (PTT)

Iron metabolism

- Ferritin

Serology

Titers assessed as positive or negative for each parameter.

- Anti HIV1/2 antibodies
- HBs-antigen
- Anti-HBc-IgG
- Anti HCV antibodies
- CMV antibodies
- Varicella zoster virus antibodies
- EBV antibodies
- HHV-6-antibodies
- HTLV 1/2 antibodies

- Treponema pallidum antibodies
- Toxoplasmosis antibodies

PCR analyses

- HIV (blood)
- HCV (blood)
- CMV (blood)
- HHV6 (blood)
- ADV (blood)
- EBV (blood)
- BK virus (urine)
- ADV (stool)

AB0 Rh blood typing

High-resolution HLA-typing and confirmatory typing

Hemoglobin Genetic Analysis

Donor specific Antibodies (DSA)

assessed by either

- Cell-based crossmatched assays (Complement-dependent cytotoxicity; CDC) or
- Flow cytometry crossmatch test or
- Solid-phase immunoassays (SPI) or
- Modified SPI such as C4d and C1q assays

Whichever method the participating center is experienced in.

Split chimerism (FACS/PCR)

The following blood samples will be collected at the transplantation center after the donor's signing of the study specific informed consent:

- **12 mL** EDTA blood for:
    - DNA analysis ('Fingerprint' sample for PCR chimerism)
    - 'Fingerprint' sample for FACS chimerism for **haploidentical donors** only

Samples will be labeled with the following details:

- *Study number*
- *Site number*
- *'Donor of' patient number*
- *Date of sampling*

Sample shipment

For sample handling and shipment please refer to **B.13.**

5.2.3. Recipient

Baseline assessments may be performed latest 4 weeks prior to start of conditioning. Before performing any study-related assessments, the investigator will inform the patient both verbally and in writing about all aspects of the study including potential benefit and risks associated with the participation in this study. No study- specific assessment will be performed unless the patient has given her/his written informed consent. Baseline assessments can be performed during a 14 days period as necessary for logistical reasons and the patient's comfort and convenience, respectively.

Note: Study related examinations needed for evaluation of inclusion/exclusion criteria (e.g. CT, MRI) which have been performed on the patient recently do not have to be repeated for baseline assessment.

Instead previous results obtained up to **3 months** prior to the baseline assessments may be used according to the investigator's judgment and if agreed by the patient.

Written informed consent has to be obtained at Visit I. For children an age-related informed consent/assent will have to be signed as appropriate, furthermore written informed consent of the child's legal representative has to be obtained. The date of consent will be recorded in the eCRF.

When the patient has consented to participate in the study, the following parameters will be assessed at the baseline visit (see *section 5.2.3.2*.).

5.2.3.1. Recipient baseline evaluation

I. Demographic characteristics

The following demographic details will be documented for each patient at baseline:

- Height in cm
- Weight in kg
- Date of birth
- Ethnicity
- Gender
- Consanguinity
- Family history
- Social history

II. Medical history

The patient's general medical history ('medical history other than underlying disease') will be assessed and documented in the eCRF at baseline. Start and stop date of any medically relevant disease and ongoing disease have to be stated. The underlying disease will be specified as described in the inclusion criteria.

Investigators will be asked to assess the clinical significance of ongoing diseases in the context of the present study. Most important in this context is a meticulous documentation of all SCD related complications and a complete organ status with relation to the underlying disease for proper risk assessment.

Note: If a condition is assessed to be 'compromising participation in this study' the patient has to be rated as meeting one or more of the exclusion criteria and may not be enrolled!

Medically relevant previous therapies for diseases other than the underlying disease will be documented at baseline with indication, trade name, start and stop date or ongoing, and current dose if ongoing. Furthermore, previous therapies of the underlying disease will be documented, including previous radio/chemotherapy (chemotherapy: regimen and number of cycles, radiotherapy: dose and start/stop date).

III. Physical examination

Physical examination of patients will be performed at each subsequent visit until study end. Results will be documented in the eCRF per body system:

- General appearance
- Eyes
- ENT (ears, nose, throat)
- Respiratory
- Cardiovascular
- Gastrointestinal
- Urogenital
- Musculoskeletal/connective tissue
- Skin/Mucosa
- Lymphatic system
- Nervous system

and pathological findings will be recorded. Patient's body weight and the performance indices Karnofsky (adult patients, >16 years) or Lansky (paediatric patients, ≤16 years) will be assessed (*appendix B.3*).

IV. Evaluation of the pre-transplant Comorbidity Index (Sorror score)
(adult patients, only)

All adult patients should be assessed using the Sorror Comorbidity index prior to HSCT (*appendix B.4*). An equivalent tool for children is not available yet, so that the comorbidities need to be collected meticioulsly in the baseline medical history of the patient.

V. Vital signs

The following vital signs will be assessed and documented in the eCRF for patients at each visit until study end as described in the patients' study flow-chart (*appendix B.9*)

- Measurement of supine systolic and diastolic blood pressure in mm Hg
- Resting heart rate in beats/min
- Body temperature in °C (oral or equivalent)
- Body weight in kg
- Hight in cm
- Respiratory rate in breaths/min
- Oxygensaturation (if indicated)

VI. Baseline examinations

- Ultrasound examination of abdomen including kidney and liver
- CT scan of chest
- Electrocardiography (ECG)
- Cardiac Performance (LVEF) determined by MUGA or echocardiography
- Electroencephalography (EEG)
- (Pediatric) Neurology
- Cerebral MRI with Time-of-Flight (TOF) sequence
- Transcranial Doppler Examination (TCD)
- Pulmonary function test
- Renal function test
- Ophtalmology with perimetry
- Otorhinolaryngology (ORL)
- Dermatology
- Dentist
- Baseline total body bone scan (MRI) within 6 months prior to inclusion*
- Liver Iron evaluation (Ferriscan, liver biopsy et al according to institutuional guidelines) within 6 months prior to inclusion

Note: Results of additional examinations obtained up to 3 months prior to the baseline assessments may be used according to the investigator's judgment.

*A total body bone scan turned out to be useful also in patients who never presented with clinical problems where osteonecrotic areas were identified via imaging. In particular haploidentically transplanted patients develop post-HSCT engraftment pain (*Section 5.1.4.4.*). The locations can frequently be traced via the pre-HSCT bonescan. Also, for medical legal reasons bonescans prior to HSCT are useful to separate steroid induced osteonecrosis from pre-existing lesions.

5.2.3.2 Recipient laboratory panel

Laboratory standard of care assessments (see below, I.–IX.) are routine parameters for evaluation of patients prior to and post HSCT according to institutional and regulatory guidelines. These parameters will be analysed at the respective certified local clinical laboratory normally used by each study site. The analysis will be performed according to the local instructions and guidelines.

Before starting the study, every investigator will supply the Sponsor with a **list of normal ranges and units of measurement** and also with laboratory certificates.
Additional study-specific laboratory assessments of outcome parameters will be performed centrally (see below, X.).

I. Recipient baseline laboratory panel

- AB0 Rh (high-resolution blood typing!) according to institutional standards
- High-resolution HLA-typing and confirmatory typing according to institutional standards
- Hemoglobin Genetic Analysis
- Serum pregnancy test in women of childbearing potential (β-HCG)
- Donor specific Antibodies (DSA) according to institutional standards

II. Routine Panel

Laboratory analyses of the 'routine panel' should be performed daily from Day 0 to Day 28 and at each subsequent visit from Day 35 to Day 100 (Visit VIII to Visit XIV). They comprise the following parameters:

Complete blood count

- Hemoglobin
- Hematocrit
- Erythrocytes
- Leukocytes
- Thrombocytes

Differential Blood count (after engraftment)

Reticulocytes (after engraftment)

Electrolytes

- Sodium
- Chloride
- Potassium

Substrate

- Glucose
- Total bilirubin
- Conjugated bilirubin
- Unconjugated bilirubin
- Creatinine
- Urea (BUN)
- C-reactive protein (CRP)

Enzymes

- Alanine-aminotransferase (ALAT)
- Aspartate-aminotransferase (ASAT)
- Gamma-glutamyl transferase (GGT)
- Lactate dehydrogenase (LDH)

Immunosuppressive drug levels (FK506) should be performed according to clinical relevance, at least twice per week!

Note: It is expected that around the time of conditioning and HSCT laboratory values of the routine panel will be outside the normal ranges for an extended period of time. This is no study-specific feature but is an expected consequence of any HSCT. All abnormal laboratory values have to be assessed by the investigator regarding clinical significance in this context. All abnormal laboratory values, which according to the investigator's judgment are clinically significant in this specific therapeutic setting have to be recorded as AEs.

III. Small Panel

Laboratory analyses of the 'small panel' should be performed **weekly** during inpatient care and at each visit from during outpatient management **in addition to the routine panel**. They comprise the following parameters:

Clinical chemistry

Electrolytes
- Magnesium
- Calcium
- Phosphate

Substrates
- Total protein
- Uric acid

Enzymes
- Creatine kinase (CK)
- Amylase
- Tryglyzeride

Quantitative viral PCR Analysis
a. CMV (blood, urine)
b. ADV (blood, stool)
c. BKV (blood, urine)
d. EBV (blood)
e. HHV6 (blood)
f. Aspergillus antigen

During outpatient care viral PCR analysis should continue weekly until CD4 counts are stably >100 cells/µl and patients are off prophylactic immunosuppression or not under immunosuppressive therapy for GVHD

Coagulation
- INR
- Fibrinogen
- Prothrombin time (PT, Quick)
- Partial thromboplastin time (PTT)

Total Chimerism Analysis
PCR-analysis of peripheral blood samples collected weekly starting with the day of engraftment until day 100, biweekly until Day 180 followed by monthly until 12 months post-HSCT compared to samples from donor and recipient collected prior to HSCT (excluded from this procedure is the time around weaning of immunosuppression and patient presenting with a mixed chimerism <90%).

Note: It is expected that due to the conditioning therapy and after hematopoietic stem cell transplantation laboratory values will be outside the normal ranges for an extended period of time. This is no study-specific feature but expected consequence of any HSCT. All abnormal laboratory values, which according to the investigator's judgment are clinically significant in this specific therapeutic setting have to be recorded as AEs.

IV. Big Panel

Laboratory analyses of the '**big pane**l' will be performed **at baseline**.

The '**big panel'** consists of the '**routine panel**' **+** '**small panel**' **+ additionally**:

Iron metabolism
- Ferritin

Hematology
- Manual differential count (as applicable)
- Hgb-Electrophoresis

Hormones
- Thyreoidea-stimulating hormone (TSH)
- T3
- fT4

- HbA1c
- Cortisole
- ACTH
- Parathormone
- Testosterone
- Prolactin
- Luteinizing hormone (LH)
- Follicle stimulating hormone (FSH
- Testosterone (male only)
- Sex hormone-binding globulin (SHBG)
- Free androgen index =100 x total Testosterone/SHBG)
- Estradiol (female only)
- Anti-Müllerian Hormone (AMH)
- Inhibin B
- Prolactin
- Insulin-like growth factor-1 (IGF-1)
- Insulin-like growth factor binding protein-3 (IGFBP-3)
- Gonadotropin releasing hormone test (GnRH Test)

Substrate
- Serum electrophoresis
- Conjugated bilirubin
- Triglycerides
- Total cholesterol, LDL and HDL

Enzymes
- Lipase

Coagulation
- Prothrombin time (PT, Quick)
- Partial thromboplastin time (PTT)
- Antithrombin (AT-III)
- Protein C
- Protein S
- APC-sensitivity (in case of levels below the lower limit, perform genetic analysis for F V Leiden mutation)
- Thrombophilic screening in case of positive family history (Cardiolipin antibodies, beta2-glycoprotein, Lupus test (DRVVT, IsPTT), Lupus anticoagulant, F VIII)

Immunochemistry
- IgG
- IgM
- IgA
- IgE

Transfusion medicine
- Hemolysins
- Coombs test
- Anti-Thrombocyte antibodies

Serology

Serology will be analysed at baseline, only. Parameters will be assessed as positive or negative.

- Anti HIV1/2 antibodies
- HBs-antigen
- Anti-HBc-IgG
- Anti HCV antibodies
- CMV antibodies
- HSV antibodies
- Varicella zoster virus antibodies
- EBV antibodies
- HHV-6-antibodies
- HTLV 1/2 antibodies
- Treponema pallidum antibodies
- Toxoplasmosis antibodies
- Chlamydia psitacci and pneumoniae antibodies
- Candida and Aspergillus
- Measels antibodies
- Mumps antibodies
- Rubella antibodies
- Diphteria antibodies
- Tetanus antibodies
- Respiratory viruses PCR by swab (Influenza, Parainfluenza, Respiratory syncytial virus)

V. Split chimerism and immune reconstitution panel (FACS/PCR)

The following blood samples will be collected at the transplantation center after the donor's signing of the study specific informed consent:

- **18 mL** EDTA blood for:
    - DNA analysis ('Fingerprint' sample for PCR chimerism)
    - FACS based immunophenotyping and immune reconstitution
    - 'Fingerprint' sample for FACS chimerism for **haploidentical donors** only

Samples will be labeled with the following details:

- *Study number*
- *Site number*
- *'Donor of' patient number*
- *Date of sampling*

Sample shipment

For sample handling and shipment please refer to **B.13.**

For complete overview of all trial-related blood sampling pleased see flow chart (*appendix B.9*)

5.2.4. Concomitant medication

Concomitant medication will be recorded from baseline until Day 180. During the follow-up phases concomitant medication will be recorded on the SAE form in case of an SAE. Furthermore, new treatment with cellular products (erythrocytes, thrombocytes or virus-specific T cells) as well as inclusion in another clinical study will be documented until the end of the study.

5.2.5. Outcome parameters

Safety/tolerability, feasibility and other outcome parameters are defined and will be measured as described in the following:

Safety parameters

I Graft-versus-host disease (GVHD):

- Incidence of aGVHD grades III–IV is defined as primary study objective. It will be expressed as 'incidence of aGVHD grades III–IV' and 'time to occurrence of aGVHD grades III–IV'.
- Incidence of aGVHD grade I-II on Day 100 post-transplantation is defined as secondary study objective. It will be expressed as 'incidence of aGVHD grade I-II' and 'time to occurrence of a GVHD grade I-II'.
- Incidence and severity of cGVHD

Statistical stopping guidelines referring to incidence of aGVHD within 100 days after SCT have been defined to ensure patients' safety throughout the study (see *section 7.11.*).

- Incidence and severity of aGVHD will be graded according to the MAGIC Criteria (*appendix B.1*: MAGIC Criteria for grading of aGVHD)
- Incidence and severity of chronic GVHD will be graded according to the NIH consensus criteria for grading of cGVHD (*appendix B.2:* Grading of cGVHD).

Note: The occurrence of acute and chronic GVHD is part of the composite endpoint EFS of the trial. Therefore, the diagnosis of GVHD should be confirmed by biopsy of the organ involved if patient fails first-line-treatment. In case of rising HHV6 titers, any skin rash should be considered worth a biopsy in order to differentiate between aGVHD and HHV6. Each biopsy will be assessed and verified by a reference pathologist and an expert panel.

II Transplant-related mortality (TRM):
TRM is defined as death occurring in a patient between day of first transplantation (Day 0) and day of assessment (all visits throughout the study), not due to underlying disease and considered related to treatment by the investigator.
Statistical stopping guidelines referring to incidence of TRM within 100 days after SCT have been defined to ensure patients' safety (see *section 7.11*.).

III Graft failure
Primary GF is defined as ANC $<0.5 \times 10^9$/L by Day 28 and platelets $<20 \times 10^9$/L (Hemoglobin <8 g/dL is omitted due to inclusion of donors with SCD heterozygosity)

Secondary GF is defined as ANC $<0.5 \times 10^9$/L after initial engraftment not related to infection, or drug toxicity, unresponsive to growth factor therapy and/or other intervention until 12 months post-HSCT.

Feasibility parameters

IV. Engraftment:

- Neutrophil cell counts will be determined by flow cytometry and time to neutrophil engraftment will be measured by determining the first of three consecutive measurements of ANC ≥500/µL following conditioning regimen induced nadir, starting from the day of the first HSCT (Day 0) until Day 28.
- Platelet counts will be determined by flow cytometry and time to platelet engraftment will be measured by determining the first of three consecutive measurements of platelet count ≥20,000/µL without platelet transfusion support for seven days, starting from the day of the first HSCT (Day 0) until Day 28.

V. Survival:

- Overall survival rate (OS) is defined as time from transplantation to death or last follow-up and will be assessed throughout the trial.
- Disease-free survival (DFS) is defined as the minimum time to recurrence, to death or to the last follow-up, from the time of transplantation and will be assessed throughout the trial.

VI. Transfusion requirement:

- Number of thrombocyte infusions needed after transplantation and time to last thrombocyte infusion starting from Day 0 until Day 100.
- Number of erythrocyte infusions needed after transplantation and time to last erythrocyte infusion starting from Day 0 until Day 100.

- Number of infusions of other blood products needed after transplantation and time to last infusion of other blood products starting from Day 0 until Day 100.

VII. Hospitalization/rehospitalization:
Number of days that patients had to be hospitalized until discharge after transplantation, and after any subsequent occurrence of an event leading to rehospitalization assessed at Day 28, Day 100 and after 1 and 2 years.

VIII. Quality of life:
Patients will be asked to answer EQ-5D (patients ≥18 years) or PedsQL 4.0 (patients <18 years) and FACT-BMT (patients ≥18 years) quality of life questionnaires at baseline, at Day 100 and 180 and after 1 year and 2 years. Results will be assessed by determining respective total score values (see *section 5.1.5.*).

Labor parameters

IX. Cell chimerism after transplantation

Total chimerism:
Total chimerism will be assessed at each study center's local laboratory by PCR- analysis of blood samples starting with engraftment.

Recommended time intervals for total chimerism analyses are:
PCR-analysis of peripheral blood samples collected weekly starting with the day of engraftment until day 100, biweekly until day 180 followed by monthly until 12 months post-HSCT compared to samples from donor and recipient collected prior to HSCT (excluded from this procedure is the time around weaning of immunosuppression and patient presenting with a mixed chimerism <90%)

Chimerism analyses from **bone marrow are not** routinely performed but are part of the trial on Day 100 and Day 240 in patients with persistent mixed chimerism. Additional sampling might become necessary for the management of peripheral blood mixed chimerisms and assessment of peripheral blood split-chimerism analyses.

For split chimerism analysis see *section 5.1.4.4.7*. and *10.1.*

X. Immune cell phenotyping and reconstitution

Immune cell phenotyping should be performed locally:
$CD3^+$, $CD4^+$, $CD8^+$, $CD3^+CD56^+$, $CD3^+TCR\alpha\beta$, $CD3^+TCR\gamma$ T cells, naive $CD4^+TCR\alpha\beta$, memory $CD4^+TCR\alpha/\beta^+$, naive $CD8^+TCR\alpha\beta$, memory $CD8^+TCR\alpha\beta$, DN $TCR\alpha\beta$, B-cells, NK cells

Centralized immune reconstitution:
Centralized immune cell phenotyping and flow chimerism will be performed as outlined in B.13 in patients who consented accompanying reseach projects.

Sample shipment
For sample handling and shipment see **B.13.**

5.3. Study visit schedule

The present study is an open label, prospective, multicenter phase II clinical study investigating the effects of TCRαβ and CD19 depleted hematopoietic stem cell grafts from G-CSF mobilized donors in the treatment of paediatric and adult patients suffering from SCD and with an indication for a HSCT transplantation. Patients in the experimental arm will receive the TCRαβ and CD19 depleted PBSC grafts as stem cell transfusion. Patients with a MSD will be enrolled in the reference arm R.
Patients will be required to follow all visits during the study according to institutional guidelines and trial-specific follow-up visits. Most if not all study-visits coincide with the usual post-HSCT follow-up visits. The **minimum** number of trial-specific post-HSCT visits are 9, scheduled at Days 30, 60, 90, 100, 180, 240, 360, 18 months and 24 months post-HSCT.

5.3.1. Donor visit schedule

Baseline Visit

Donors will have to visit the transplantation center

- For general information about risks and benefits of donation
- To give their study specific informed consent
- For collection of study-specific reference blood samples (see *section 5.2.2*). This visit will have to be scheduled at ≤28 days prior to the first administration of medication for stem cell mobilization at the collection center. (Note: treatment with stem cell mobilizing medication and apheresis will be performed according to medical routine for stem cell donation and not part of the study-specific procedures).
- After donation donors will have to come to the transplantation center for follow-up visit(s) according to institutional guidelines and regiulations.

5.3.2. Patient visit schedule

Baseline (within four weeks prior to start of conditioning, Day -40 to Day -14)

Visit I, excluded are the screening visits for consultation and patient enrolment.

Conditioning (Day –12 to –1)

- Visit II

Transplantation and Inpatient Care (Day 0 to Day 28)

- Visit IIIa (Day 0 - 2): Stem Cell Infusion (Transplantation)
- Visit IIIb (Day 2 - discharge): Engraftment, Reconstitution and Discharge

Post-transplantation phase I (Day of discharge to Day 180)

- Visit IVa – d Day at first visit **after discharge** (equivalent to Day 30)**, therafter weekly:**, assessment of stable engraftment, PB chimerism, aGVHD and AEs
- Visit V Day 60: Assessment of stable engraftment, PB chimerism, aGVHD and AEs. Additionally, centralized immune reconstitution and split chimerism (PB)
- Visit Va Day 80: Optional split chimerism (PB) analysis for patients with mixed chimerism
- Visit VI Day 100: PB chimerism + BM chimerism, split chimerism (PB + BM), aGVHD and AEs, centralized immune reconstitution
- Visit VII Day 180: peripheral blood, a/cGVHD, AEs, split chimerism (PB), centralized immune reconstitution

Post-transplantation phase II (Day 180 for MSD and 240 for haploidentical HSCT to Day +360, 18 months and 2 years)

- Visits VIII Day >180: **weekly** PB chimerism analyses with start of weaning of immunosuppression in MSD, cGVHD, AEs
- Visits IX > Day 240: **weekly** PB chimerism analyses with start of weaning of immunosuppression in haploidentical HSCT, cGVHD, AEs, split chimerism (PB + BM) in patients with mixed chimerism only!
- Visit X Day 360: PB chimerism, cGVHD, AEs, split chimerism (PB), centralized immune reconstitution
- Visit XI at 18 months: PB chimerism, cGVHD, AEs
- Visit XII 24 months: study close-out visit

Follow-up phase II: after 24 months

- Additional follow-up visits after month 24 if indicated due to unstable mixed chimerism, cGVHD, lack of immune reconstitution. Post-transplant care according to institutional guidelines and regulations including vaccination.

5.4. Unscheduled visits

It is at the discretion of the investigator to appoint additional visits if medically indicated. Measures and assessments performed and the date and reason for such a visit have to be documented in detail in the eCRF.

5.5. Early termination visit (ETV)

If a patient prematurely discontinues the study because of any reason before Day 100 all assessments planned for the regular Visit VI (Day 100) should be performed at an early termination visit ('Early Termination Visit [ETV] A'). The following examinations have to be performed at the ETV A:

Examinations

- Physical examination, including Karnofsky/Lansky performance status
- Vital signs
- Disease status (Status of SCD related complications)

Laboratory standard of care

- Laboratory assessments, 'small panel' (see *section 5.2.3.2, III.*)
- Collection of blood sample for special laboratory analyses (Hb Electrophoresis)
- Collection of blood sample for PCR analysis of aspergillus, CMV, ADV and EBV infections
- Bone marrow puncture for laboratory staging (only in cases with a mixed chimerism or if indicated otherwise)

Adverse events and concomitant medication

- Documentation of AE/SAE
  Note: Infections which are not life-threatening will be documented in the eCRF as SAE but will not be recorded on special SAE report forms on paper and will not be reported as SAE irrespective of duration of hospitalization.
- Documentation of concomitant medication

Outcome parameters: safety

- Assessment of aGVHD and cGVHD
- Monitoring of TRM

Outcome parameters: feasibility

- Monitoring of OS and DFS
- Monitoring of occurrence and duration of rehospitalization
- Completion of quality of life (EQ-5D or PedsQL and FACT-BMT) questionnaires

Outcome parameters: laboratory

- Collection of samples of peripheral blood and of bone marrow, if indicated, for analyses of chimerism
- Collection of blood samples for analysis of reconstitution of the immune system.

If a patient prematurely discontinues the study because of any reason **after Day 100** all assessments planned for the regular Visit IX (Month 12) should be performed at an 'Early Termination Visit [ETV] B'. The reason for premature discontinuation has to be documented in the eCRF. The following examinations have to be performed at the **ETV B**:

Examinations

- Physical examination, including Karnofsky/Lansky performance status
- Vital signs
- Disease status (Status of SCD related complications)

Laboratory standard of care
- Laboratory assessments, 'small panel' (see s*ection 5.2.3.2, III*.)
- Collection of blood sample for PCR analysis of CMV, ADV and EBV infections

Serious adverse events
- Documentation of SAE (concomitant medication will be documented in case of an SAE on the SAE form)
  Note: Infections which are not life-threatening will be documented in the eCRF as SAE but will not be recorded on special SAE report forms on paper and will not be reported as SAE irrespective of duration of hospitalization.
- Documentation of new treatment with cellular products and inclusion in other clinical study.

Outcome parameters: safety
- Assessment of aGVHD and cGVHD
- Monitoring of TRM

Outcome parameters: feasibility
- Monitoring of overall and disease-free survival
- Monitoring of rejection/GF rate
- Completion of quality of life (EQ-5D or PedsQL and FACT-BMT) questionnaires

Outcome parameters: laboratory
Collection of blood sample for immune cell phenotyping.

6. ADVERSE EVENTS

6.1. Definitions

6.1.1. Adverse event (AE)

The term adverse event describes any untoward medical occurrence in a patient or clinical investigation subject administered an investigational medicinal product (IMP). It does not necessarily have a causal relationship with this study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product (IMP).

The methods for assessing safety parameters consist of clinical routine methods (physical examination, vital signs, laboratory and clinical evaluation) and measures for observation of patients after haploidentical HSCT according to institutional and regulatory guidelines. Laboratory testing will include analysis for recurrence or newly occurring infectious diseases caused by CMV, ADV, HHV6, BKV and EBV and aspergillus.
Therapy-related toxicities are expected during the conditioning period and will be rated as known and unknown therapy-related toxicity. Unknown therapy-related toxicities will have to be documented as adverse event as described in *section 6.1.1*. From Day 0 onwards no further distinction between known and unknown therapy-related toxicities will be made. All AEs will be documented from Day 0 until 24 months post-HSCT. SAEs as defined in *section 6.1.3.* will be documented for patients in the eCRF from the baseline visit until the end of study.
AEs will be recorded in the eCRF and SAEs will be recorded on special SAE case report forms on paper. Safety outcome parameters (GVHD, TRM and infusional toxicity) will be assessed as described below (see *section 5.2.5*).

This definition includes:
- Any sign or symptom occurring during the study
- Any accident resulting in an untoward medical occurence
- Any significant change in laboratory parameters

Pre-existing conditions or diseases present on the day of enrolment in the study should be documented as AEs only if symptoms worsen in severity or increase in frequency during the study. A pre-existing condition that remains unchanged from baseline is not an AE.

AE exclusions

In the context of this study, the following event is excluded from all AE reporting:

- Known therapy-related toxicities occurring prior to **discharge post-HSCT**

For this exclusion, "therapy-related" refers to the conditioning treatments applied during the condition phase that is used to prepare for HSCT.

6.1.2. Adverse reaction (AR)

Adverse reactions (ARs) include all untoward and unintended responses to an Investigational Medicinal Product (IMP) related to any dose administered. This covers also medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

This definition also includes:

- Any reaction to drug withdrawal
- Any drug interaction resulting in an untoward medical occurrence
- Any effect related to overdose, abuse or dependence.

All AEs judged by either the reporting Investigator or the Sponsor as having a reasonable possibility of a causal relationship with the IMP qualify as ARs. This means that there are facts (evidence) or arguments to suggest a causal relationship between the event and the IMP. An AR is defined as unexpected when its nature, severity or outcome is not consistent with the reference safety information provided in the Investigator's Brochure (IB). Note that "severity" is used to describe the intensity of the specific event, not the seriousness.

6.1.3. Serious adverse events (SAEs)

Serious Adverse Event (SAE) is any untoward medical occurrence or effect that at any dose:

- results in death,
- is life-threatening,
- requires **unexpected*** hospitalization **after initial discharge post-HSCT**
- or **unexpected*** prolongation of existing hospitalization,
- or **unexpected*** admission to the intensive care unit **during** inpatient care
- results in persistent or significant disability/incapacity,
- is a congenital anomaly or birth defect
- is an important medical event that may not immediately be fatal, life-threatening or require hospitalization but, based upon appropriate medical judgment, is thought to jeopardize the subject or require an intervention to prevent one of the outcomes listed above.

***Unexpected is defined as an unusual event outside a known or expected therapy-related toxicity occurring after Day 0.**

The serious criteria listed above have to be considered at the time of the event. For example, a life-threatening event is an event in which the subject is at risk of death at the time of the event; it does not refer to an event which hypothetically might cause death if it were more severe.

Please note:

- A planned hospitalization for a pre-existing condition or hospitalization for a procedure required as per the study protocol is not an SAE.

A pregnancy detected during the study is not an SAE, but should be reported immediately to the Sponsor as described in *section 6.6* below.

Exemptions from expedited SAE reporting

In the context of this study (seriously ill patients undergoing conditioning treatment leading to immune dysfunction), the following events meeting the above definition for an SAE will be documented in the eCRF as SAE, but will **not require expedited reporting on paper SAE report forms**:

- An infection requiring inpatient hospitalization or prolongation of hospitalization, which is not life-threatening or fatal
- A known or expected therapy-related toxicity occurring after discharge requiring inpatient hospitalization or prolongation of hospitalization, which is not life-threatening or fatal.

6.1.4. Serious adverse reactions (SARs) and suspected unexpected serious adverse reactions (SUSARs)

An AR that fulfills one or more seriousness criteria, as defined above, is a serious adverse reaction (SAR). A suspected unexpected serious adverse reaction (SUSAR) is a SAR, the nature or severity of which is not consistent with the reference safety information contained in the IB.

Events which add significant information on the specificity, increase of occurrence, or severity of a known, already documented SAR constitute unexpected events. For example, an event more specific or more severe than described in the IB is to be considered "unexpected".

As per the Clinical Trial Facilitation Group Reference Safety Information Q&A document published in November 2017, all **life-threatening or fatal SARs** will be treated as **unexpected** and classified as SUSARs, irrespective of whether previous life-threatening or fatal reactions have previously occurred.

6.2. Assessment of adverse events/therapy-related toxicities

6.2.1. Severity

The severity of AEs must be assessed using the categories: mild, moderate and severe. This assessment is subjective and medical judgment should be used to compare reported AEs with similar types of events observed in clinical practice. It is important to recognize that severity is not equivalent to event seriousness. Guidelines for severity assessments are listed below:

- **Mild**: Awareness of a sign or symptom barely noticeable to the patient or does not make the patient uncomfortable; the AE does not cause a limitation of the usual activities.
- **Moderate**: Symptom with enough discomfort to cause interference with normal activities. Treatment of symptom may be needed.
- **Severe**: Symptom of a sufficient severity to cause the patient severe discomfort and prevent performance of normal activities; resistant to conventional symptomatic treatment.

6.2.2. Causality

The causality of each AE must be evaluated by an Investigator and documented in the eCRF. Investigators will categorise events as 'IMP-related', 'concomitant medication-related', 'other' or 'unrelated'. When assessing a potential causal relationship to study treatments and/or procedures, a simple binary decision will be made according to the following criteria:

Not related: No reasonable possibility of a causal relationship.
Related: A reasonable possibility of a causal relationship. This means that there are facts (evidence) or arguments to suggest a causal relationship.

Every SAE that is subject to expedited safety reporting will be assessed for causality to the study IMP twice, by the reporting Investigator and also independently by the Sponsor. The causality assessment given by the Investigator should not be downgraded by the Sponsor. If the Sponsor disagrees with the Investigator's causality assessment, the opinion of both the Investigator and the Sponsor will be documented.

The following points should be taken into account when assessing causality:

- Length of time between administration of the medicinal product and the onset of the adverse event
- Drug levels and evidence, if any, of overdose
- A known or expected response pattern to the suspect medicinal product, including previous experience with the product and whether the adverse event is known to have occurred with the product
- Physiological effects of the medicinal product
- Known adverse events related to medicinal products belonging to the same or a similar class, if explained by the pharmacological action or with regard to findings in animals or a specific genetic predisposition of the patient.

6.3. Monitoring, recording and reporting of adverse events

6.3.1. General requirements

The Investigator will be responsible for ensuring that correct information is provided in the eCRF and on the SAE Form.

The following data will be recorded:

- Description of the AE: nature, frequency, intensity, time (if within 24 hours of HSCT application) and date of onset and resolution, outcome, and causal relationship according to the Investigator
- Whether the AE is serious (SAE)
- Action taken

All AEs occurring during the study, irrespective of the suspected causality, will be monitored:

- until the event has completely resolved or stabilized, or in case of a clinically significant abnormal laboratory value has returned to baseline or stabilized at an acceptable level according to the Investigator,
- until there is a satisfactory explanation for the changes observed, or
- until the patient is lost to follow-up.

The institutions/physicians following the patients during and after convalescence will be informed about the patient's study participation and advised to be vigilant to safety-related events. The study center Investigator will conduct safety-related follow-up examinations on an outpatient basis for a minimum of 6 months or until resolution of SAEs ongoing at Visit XI (24 months post-HSCT).

In case of withdrawal, the respective eCRF has to be completed. The results of additional diagnostic measures as the result of an AE, such as laboratory tests, ECG, angiogram, echocardiogram and MRI must be available at site.

6.3.2. Reporting period

AEs will be reported in the eCRF throughout the trial from Day 0 until the end of the study (24 months post-HSCT). Unknown therapy-related toxicities during the conditioning period (Day -12 to Day -1) should also be documented as AEs. From discharge onwards no further distinction between known and unknown therapy-related toxicities will be made.

SAEs will be reported in the eCRF from baseline until the end of the study (24 months post-HSCT). SAEs subject to expedited reporting on paper will be reported from Day 0 until the end of the study. Expedited reporting on the SAE Form is not required prior to Day 0, since an assessment of the relationship to IMP cannot be made at this time (IMP is given on Day 0).

6.3.3. Reporting of serious adverse events

During the course of the study, SAEs will be recorded in the eCRF. Additionally, the Investigator has to immediately report to the Sponsor (within 24 hours of awareness) all SAEs that are subject to **expedited reporting**. These SAEs will be documented on the paper SAE Form and sent via fax transmission to the safety team:

Pharmacovigilance Safety Desk **Fax: +49 941 944-6772**

Complete information on an SAE may not be available at the time of the initial report. In case of incomplete information, the Investigator must report further relevant information immediately after awareness as follow-up reports. In certain cases, it may be appropriate to submit several follow-up reports before a complete and final evaluation of the entire case is possible.

The initial and follow-up reports must identify the clinical trial participants by participant identification number.

Note: Events that are exempt from expedited SAE reporting (see *section 6.1.3* above) do not need to be reported on the paper SAE form and faxed to the safety team. These events will be documented in the eCRF only.

6.3.4. Reporting of SUSARs by the sponsor

The sponsor will report all relevant information concerning SUSARs to all concerned national competent authorities, ethics committees and Investigators within the following time lines:

- **Fatal and life-threatening SUSARs**: No later than 7 days after knowledge, with relevant follow-up information provided within an additional 8 days

All other SUSARs: No later than 15 days after knowledge

6.3.5. Annual safety reporting by the sponsor

Once a year throughout the clinical trial, the Sponsor will create and submit to the concerned national competent authorities and ethics committees an annual safety report that follows the format and conventions of the Development Safety Update Report (DSUR) described in ICH Topic E2F. The DSUR will contain a listing of all serious adverse reactions (SARs) that have occurred during the one-year period and a cumulative summary of all SAEs.

6.3.6. Other safety issues

Events may occur during a clinical trial that do not fall within the definition of SAE/SUSAR and thus are not subject to the reporting requirements for SAEs/SUSARs, even though they may be relevant in terms of subject safety. Any safety issues that might alter the current risk-benefit assessment of the IMP, whilst not falling within the regulatory definition of SAE or SUSAR, will nevertheless be reported to the concerned competent authorities and ethics committees by the sponsor.

6.4. Adverse events of specific interest

GVHD grade III and grade IV, TRM and graft failure are events of specific interest but are already part of the composite primary endpoint and will therefore be monitored.

Additional AEs of **specific interest** are the so called transplant-related endotheliopathies that might be observed more frequently in the patient population studied. They are therefore of particular interest for the safety of this study and will be monitored in detail.

Adverse events of **specific interest**

- Transplant-related neurotoxicity (including PRES) (*section 5.1.4.4.8.*)

- Macrophage-activation syndrome (mentioned in (*section 5.1.4.4.6.*)
- Diffuse alveolar hemorrhage
- SCD related Engraftment syndrome (*section 2.5.4.8.*)
- Sinusoidal obstruction syndrome/veno-occlusive disease (SOS/VOD) *(section 2.5.4.6.*)

Adverse events of specific interest as described above will be continuously documented until Visit XI, 24 months post-HSCT and have to be reported outside of the regular SAE reporting framework in a dedicated form. Adverse events of specific interest will be reported to the DSMB. Details of the DSMB's responsibilities and working procedures are provided below, see section *9.4.*

6.5. Therapy-related toxicities of conditioning

Therapy-related toxicities are known during the conditioning period and will have to be rated as known and unknown therapy-related toxicity. Unknown therapy-related toxicities will have to be documented as AE in the eCRF. From discharge onwards no further distinction between known and unknown therapy-related toxicities will be made. Known therapy-related toxicities are to be found in the respective SmPCs. Adverse events will be documented from Day 0 to the end of the study (24 months post-HSCT).

6.6. Pregnancy

Pregnancy is an exclusion criterion for this clinical trial. Accordingly, contraceptive measures are obligatory in woman of childbearing potential throughout the study.
Any pregnancy detected during the study (either in a trial participant or an individual impregnated by a trial participant) should be reported immediately via fax to the safety team at the Sponsor using the dedicated pregnancy report forms:

Pharmacovigilance Safety Desk **Fax Number: +49 941 944 6772**

Any pregnancy must be followed to term. The outcome for mother and child has to be documented and reported to the Sponsor on the relevant pregnancy outcome form. A pregnancy may also produce an SAE, if it results in a congenital anomaly or birth defect. In this case, a separate SAE should be reported immediately as per the SAE reporting guidelines described above. Any SAE relating to a congenital anomaly or birth defect will be assessed for its relationship to the study IMP and investigated thoroughly by the sponsor.

6.7. Unblinding of treatment / emergency identification

Not applicable.

7. STATISTICS

7.1. General aspects

This trial is a multicenter, non-blinded, two-armed stratified, prospective clinical phase II trial. All analyses will be done according to the "intention-to-treat" principle. Per-protocol analyses will be performed for explorative reasons. Descriptive measures for all variables will be given: Absolute and relative frequencies for categorical data; minimum, maximum, median, quartiles, mean, standard deviation, and skewness for continuous outcomes; Kaplan Meier curves and quartiles of the survival times with the 95% confidence intervals (CI), and survival rates at 1, 2, and 3 years with the 95% CI for survival data; Hazard ratios will be given with 95% CI. Unless otherwise specified below, unpaired samples will be compared with respect to a categorical / continuous / time-to-event outcome using Fisher's exact test / Mann-Whitney U test or Kruskal-Wallis test (in case of two or more than two samples) / log-rank test.
The objective of this phase II trial is to prove that EFS following T-Haplo-SCT (experimental group E) is non-inferior to matched sibling donor (MSD) HSCT (reference group R). Typically, phase II-trials are

single-arm trials testing an experimental treatment against a historic reference. However, 'historic' data on MSD HSCT are not uniform enough to be used as unbiased reference for T-Haplo-SCT in SCD. Thus, group R will be included as prospective reference group in a two-arm design. Experimental group E (reference group R) is defined as patients with no MSD (with MSD) who will be treated with T-Haplo-SCT (MSD HSCT). Reference group R and experimental group E will thus be transplanted almost identically. In particular, treatment arm allocation is done according to availability of MSD. A design based on randomized allocation of treatment arm within the group of patients with available MSD was discarded for ethical and economic reasons, since donor availability is rare (<20%) and since the latter would imply withholding MSD HSCT for patients with available donors.

7.2. Analysis populations

Intention-to-treat (ITT) population: This population includes all trial subjects enrolled into the trial.

Per-protocol (PP) population: This population is defined by patients from the ITT-population who fulfill the inclusion and exclusion criteria and were treated conform to protocol. Deviations from the protocol including violations of inclusion/exclusion criteria will be assessed as 'minor' or 'major' based on the combined decisions of the trial monitor and the Sponsor. Patients also are considerd as treated conform to protocol if only minor protocol violations are recorded.

Safety population: This population includes all trial subjects who underwent transplantation.

7.3. Primary endpoint analysis

The primary endpoint is the event free survival (EFS) measured from HSCT until event and is defined as time from transplantation to event. Event is defined as aGVHD (Grade III - IV), cGVHD (moderate/severe), primary graft failure (pGF), or death (from any reason).
Primary null hypothesis H_0: The EFS of SCD-patients with no matched donor and treated with T-Haplo-HSCT (experimental group E) is non-relevantly inferior to EFS of the prospective reference group R (SCD- patients with MSD and treated with MSD HSCT). In formulas, H_0: $\omega \geq \omega_0$, where ω is the true hazard ratio of the experimental group E to the reference group R and where ω_0 = 1.13 is the non-inferiority margin. The null-hypothesis is tested by one-sided Wald test in a stratified Cox-regression with treatment group (experimental or reference) as binary covariate, stratified by age group (<16 or ≥16 years at transplantation), one-sided α=0.05, power 80%.
Additionally, a confirmatory confidence interval for the true hazard ratio ω will be constructed. That is, for every $\omega' \leq \omega_0$ the following null hypothesis will be tested; $H_{0,}{}'$: $\omega \geq \omega'$. Adjustment to multiple testing is done by hierarchical testing, where for every $\omega'' \geq \omega'$ null hypothesis $H_{0,}{}''$ is considered hierarchically superior to $H_{0,\omega}{}'$. In particular, null hypothesis H_0 coincides with null hypothesis $H_{0,0}$ and is thus hierarchically highest according to this ordering. For each $\omega' \leq \omega_0$, $H_{0,\omega}{}'$ is tested in analogy to H_0.This offers the possibility to assess superiority of EFS in the experimental group over EFS in the reference group without affecting type I error rate control for H_0, once non-inferiority has been proven.

7.4. Key secondary analysis

OS and DFS will be assessed by Wald test in a stratified Cox-regression with treatment group (experimental or reference) as binary covariate, stratified by age group (<16 or ≥16 years at transplantation). The impact of potential risk factors on EFS, OS and DFS will be analyzed by Cox regression models. Chi-squared test is used to correlate the frequency of a/cGvHD, secondary GF and immune- reconstitution with treatment arm (group E or R) by age stratum (<16 or ≥16 years at transplantation). In order to assess the impact of treatment arm (group E or R) by age stratum (<16 or ≥16 years at transplantation) on QOL, Mann-Whitney U test is applied. Analysis of fertility will be descriptive.

7.5. Methods against bias

Since it will be a stratified trial according to donor availability, the main source of bias is that the T-haplo group will consist of older and potentially sicker patients who have not been transplanted so far. The MSD group will in general be younger and therefore less affected by risk of rejection and GVHD. On the other hand, viral infections, in particular adenovirus will be more prevalent in this group. Since we have not experienced any significant problems to that regard in our fairly large pilot group, our concern limited about this source of bias. Also, because with the advancement of the trial, the issue of age will be balanced when the adult group is being transplanted and the younger patients are increasingly recruited. In order to nevertheless account for a potentially higher risk of rejection and GVHD in older patients, the main question is analyzed using a stratified test, adjusting for age (younger vs older), with the treatment effect, however, being expected to be consistent in younger and older patients. An adaptive interim analysis will help to adjust the sample size according to the observed treatment allocation ratios in the age strata, thus reducing the impact of potential sources of bias on power of the trial. As indicated above a blinding is not feasible since withholding a MSD HSCT in SCD patients for randomization is not ethical at this stage. Availability of a MSD is not driven by covariates like for example age that might influence the primary endpoint EFS. Therefore, treatment allocation by availability of a MSD is expected to be random and unbiased. All participating centres are highly experience academic institutions, JACIE accredited and trained in transplanting complex patients so that trial-site effects are negligible. GVHD, one of the primary outcome indices, is verified by the requirement of a confirmatory biopsy that is evaluated by expert pathologists for gut, liver and skin and a panel of independent, transplant physicians, highly experienced in GVHD.

7.6. Sample size calculations

Sample size is determined according to power requirements for the confirmatory null hypothesis HH_0 specified in *Section 7.3*. For the sake of a robust a priori sample size estimation that does not depend on specific assumptions on the treatment allocation ratios within the age strata (<16 or ≥16 years at transplantation), the initial sample size calculation is performed for an unstratified test situation. This constitutes a conservative approach since the variance of estimators is reduced by adjustment for relevant covariates. At the adaptive interim analysis, a sample size recalculation according to the conditional power approach will be performed while using the observed interim estimates (i) of the treatment allocation ratios within the age strata (<16 or ≥16 years at transplantation), (ii) of the event number ratio between the age strata, and (iii) the observed treatment effect. This realizes a refinement of the initial sample size calculation based on available interim information on initially unknown parameters. Thereby, within the bounce of sample size recalculation, the maximum sample size of 265 patients (corresponding to a total accrual period of 5 years) must not be exceeded.

Initial sample size calculation: In the unstratified setting, sample size calculation for the Cox-regression with one binary covariate reduces to that for the common two-sample log-rank test[100]. So, with the adaptive design specified in 11.3 (applied with the corresponding unstratified test statistic), under the assumption of 2-years EFS-rates 0.73 (reference intervention) versus 0.90 (experimental intervention) and a non-inferiority margin (hazard ratio: experimental vs. reference treatment) ω_0 = 1.13, the power of the first interim analysis is 20% and the power of the final analysis is 80% if the significance test of the respective analysis is performed at 9 and 27 accumulated events pooled over both therapy groups (ADDPLAN 6 MC, Version 6.0.9). In view of an expected ratio of 4:1 of patients with no suitable matched donor as compared to patients with matched donor, an allocation ratio of 4:1 (experimental vs. reference) was assumed. To estimate the required accrual period historic data were used to fit EFS-curves. A plateau of the EFS-curves after 2 years was adopted according to the data, i.e. it was assumed that statistically no events will occur after 2 years. With an accrual rate of 53 patients per year for 4 years and under the assumption of a 2-years loss-to-follow up rate of 5% (with exponentially distributed drop-out times), the interim analysis is expected to be performed after 2,25 and the final analysis within 7 years. With the adaptive design the accrual time, follow-up time, and the number of required events can be adjusted to the data of the interim analysis. The latter allows refinement of the initial sample size calculation based on interim information on initially unknown parameters.

Justification of planning assumptions: Unpublished data provided by Gluckman et al[16] suggest a 2-years EFS at 73%. In T-HAPLO-SCT, updated pilot data show a plateau of EFS-curves at 90% after 2-years. Regarding choice of the non-inferiority margin, experts were surveyed on a range of differences that

they consider to be unimportant, and the margin was chosen based upon a summary statistic of the responses (cf. EMA Guideline on the choice of the non-inferiority margin). No power analysis for the secondary endpoints was performed.

7.7. Compliance / Rate of loss to follow-up

The relation of a pediatric transplant physician and his patient/parents is mostly due to the severity of disease, very particular and trustworthy. We transplanted so far 26 patients with SCD, the rate of non-compliance is nil. This is the general experience in patients, who pass extensive preparations for HSCT and are followed for several years. Of all patients transplanted in Regensburg over the last 8 years, nobody was lost to follow-up (F/U). This is reproducible for most pediatric transplant centres. One reason for loss to F/U would be repatriation of refugees so that patients with a temporary residency permit will not be transplanted. Nevertheless, a 5% drop-out rate is incorporated in the statistical analysis.

7.8. Interim and final analysis

An adaptive design with one interim analysis is planned for the analysis of HH_0. The characteristics of the adaptive design are determined according to the inverse normal method. The bounds of the 2-step adaptive design result from a group-sequential design without futility stop according to Wang and Tsiatis[19] with boundary shape parameter Δ=0.23, one-sided α=5%, and information rates 0.33 and 1. The interim and final analysis are intended to be performed at 9 and 27 accumulated events, pooled over both arms. The trial will be stopped after the interim analysis, if the main question can already be answered. With the adaptive design the accrual time, follow-up time, and the number of required events can be adjusted to the data of the interim analysis.

7.9. Missing data

The primary aim will be analyzed by Wald test in stratified Cox-regression. Accordingly, subjects withdrawn from the trial will be treated in the canonical way as censored. Analyses of secondary aims will be complete cases analyses.

7.10. Subgroup analyses

Subgroup analyses will be performed in subgroups defined by gender and age stratum (<16 or ≥16 years at transplantation), amongst others.

7.11 Pre-defined statistical stopping guidelines (Safety)

The rate of TRM and of aGVHD (grade III – IV) will be compared between both treatment arms by one-sided tests of rates. Group sequential designs on an overall level of 10% are intended to be performed using a Pocock type [101] α-spending approach with 5 analyses (after 1,2,3,4,5 years, synchronized with the annual DSMB report) based on a maximal number of 212 trial patients pooled over both arms. Analogous analyses will be performed within each age stratum (<16 or ≥16 years at transplantation). As complementary analysis, the overall rate of TRM and aGVHD (grade III – IV) will estimated and compared between both treatment arms by Fisher's exact test on an overall level of 10% at each DSMB report. If any of the analyses shows a relevant inferiority of the experimental treatment, the result will be judged as a critical safety finding to be discussed with the DSMB.

8. ETHICAL ASPECTS

8.1. Independent ethics committee approval

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Independent Ethics Committee / Institutional Review Board (IEC/IRB). A signed and dated statement that the protocol and informed consent have been approved by the IEC/IRB must be available at the sponsor before study initiation. The name and occupation of the chairman and the members of the IEC/IRB must be present at the sponsor.

8.2. Informed consent

Patients who are eligible for enrolment into the study will be informed by the investigator in detail about the study. Donors will be informed about additional requirements (data transfer and collection and genetic analysis of one blood sample), which are needed to allow the patients' study enrolment. Information for donors does not involve routine procedures of stem cell mobilization and apheresis because donors will already have agreed to these procedures prior to being invited to donate HSC for transplantation in the context of the present study. For information about these requirements, for giving consent and for collection of the blood sample the donor and where applicable their legally authorized representative has to visit a study center once. Patients will be allowed adequate time for consideration and making an informed decision, at least 24 hours. During this period patients will have the opportunity to discuss questions and concerns with their treating physician. If patients are willing to participate in the study, informed consent will be obtained from them or their legally authorized representative according to the regulatory and legal requirements of Germany. The consent form will be dated and retained by the investigator as part of the study records. The investigator will not undertake any investigation specifically required for this clinical study until valid consent has been obtained. The date when consent was obtained will be documented in the eCRF.

According to the German Medicines Act, under-age patients personally have to give informed consent in addition to their legal representatives, provided they are able to understand the information given to them in this study.

The explicit wish of a minor or a mentally incapacitated adult, who is capable of forming an opinion and assessing the study information, to refuse participation in or to be withdrawn from the study at any time has to be considered by the investigator.

Patients/donors will be asked expressly to give their consent for use of their samples in genetic analysis (additional assessments of transplantation success). If a patient denies his/her consent to this analysis he/she cannot be enrolled in the study, because the additional assessments are obligatory for determination of study endpoints.

Patients can withdraw their consent at any time during the study period without having to give a reason and without prejudice regarding their future medical treatment.
If a protocol amendment is required, the informed consent form may need to be revised to reflect the changes to the protocol. If the consent form is revised, it has to be reviewed and approved by the appropriate IEC/PEI and signed by all patients subsequently enrolled in the study as well as those currently enrolled in the study.

8.3. Data confidentiality

All study findings and documents will be regarded as confidential. The investigator and members of his research team are not allowed to disclose such information without prior written approval from the Sponsor.

The anonymity of participating patients has to be maintained. Patients will be identified on the eCRF submitted to the Data Base by their patient number, not by name. Documents not to be submitted to the Data Base that identify the patient (e.g., the signed informed consent) must be maintained in confidence by the investigator

8.4. Liability and insurance

The insurance for study-related claims will be covered by Newline Europa Versicherung AG.

The Sponsor will take out reasonable third-party liability insurance cover in accordance with all local legal requirements. The civil liability of the investigator, the persons instructed by him and the hospital, practice or institute in which they are employed and the liability of the Sponsor with respect to financial loss due to personal injury and other damage that may arise as a result of the execution of this study are governed by the applicable law.

The Sponsor will arrange for patients participating in this study to be insured against financial loss due to personal injury caused by the IMP tested or by medical steps taken in the course of the study. If concomitant enrolment of a patient in another clinical study after Day 100 is planned according to the investigator's medical advice, the insurance companies of the present and of the new study will have to be informed accordingly before enrolment.

9. ADMINISTRATIVE PROCEDURES

By signing the study protocol, the investigator accepts to comply with all of the following points:

9.1. Regulatory aspects

The study will be conducted according to the requirements of the declaration of Helsinki (revision 1996) and in compliance with the current provisions of the German Drug Law, the respective decrees and the European Clinical Trial Directive. Regulatory reporting requirements will be agreed on by the parties in the investigator contract.

9.2. Protocol approval and amendment

Before the start of the study, the study protocol and other relevant documents will be approved by the IECs and Competent Authorities (PEI), in accordance with German legal requirements. The Sponsor must ensure that all ethical and legal requirements have been met before the first patient is enrolled in the study.

This protocol is to be followed exactly. For any alteration of the protocol, amendments must be written, receive approval from the appropriate personnel, and receive IEC and Competent Authority (PEI) approval prior to implementation if appropriate. Administrative changes not affecting the patient benefit/risk ratio may be established without the need for a formal amendment.
All amendments will be distributed to all protocol recipients, with appropriate instructions.

9.3. Duration of the study

- The time for preparation of the trial was 12 months.
- The recruitment period will be approximately 48 months.
- First patient in to last patient out will be approximately 72 months.
- The time for data clearance and analysis will be 6 months.
- The duration of the entire trial is estimated to be 84 months.

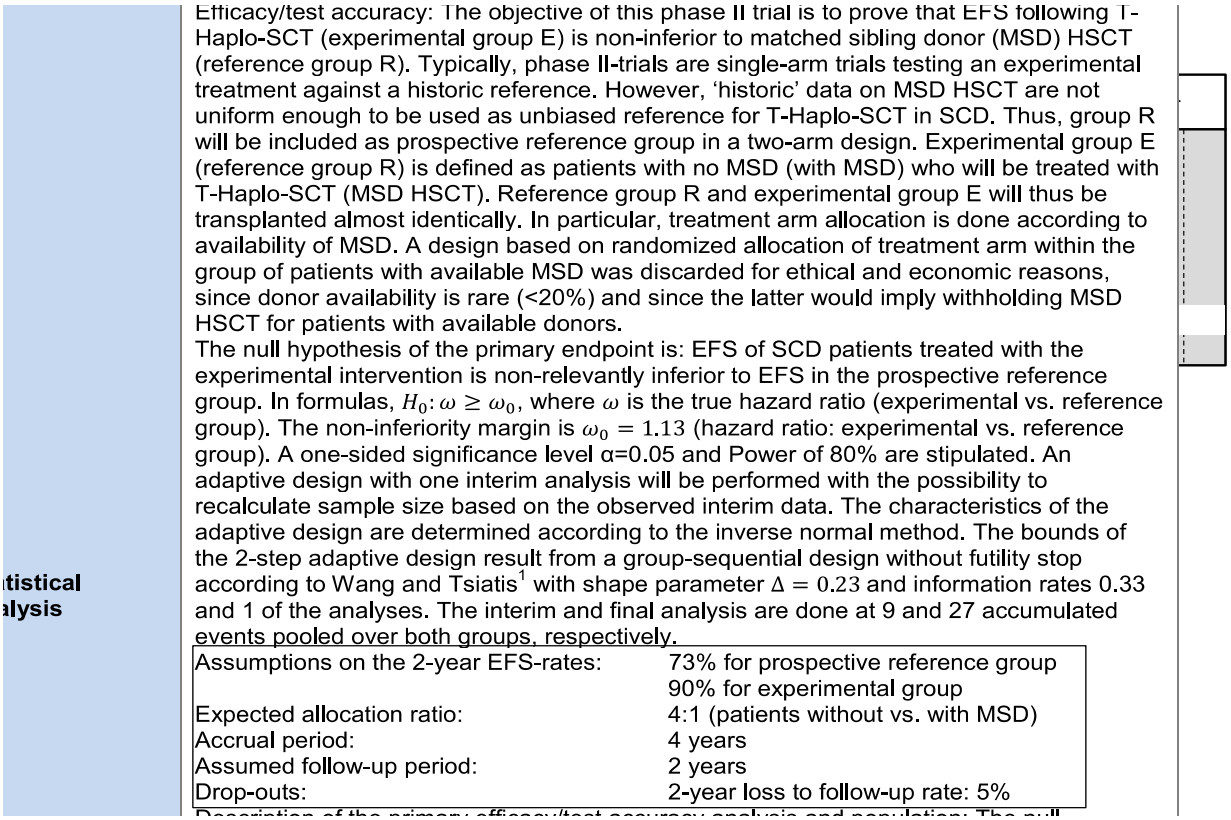


Fig. 3: Study timeline

9.4. Data safety monitoring board (DSMB)

The major task of DSMB will be to ensure well-being of patients in this trial, with special attention to life-threatening and fatal events in treatment phases. AEs and discontinuations are to be reviewed by DSMB. In cases of concern vital signs and status should be reviewed as well. During this evaluation enrolment and treatment of patients will continue. The decision-making criteria for individual patient discontinuation are presented in the individual study protocols and are not the purview of the DSMB.
The duty of the DSMB is going to advise the Principal Investigator (PI) regarding potential safety signals that may or may not appear in the data reviewed. The recommendations of the DSMB ("DSMB letter, minutes, and recommendations") are to be send to the PI for the T-Haplo for SCD trial and distributed as per paragraph 5. The PI would then discuss with the Advisory Board (AB) Members and Drug Safety (DS) in conjunction with others (see paragraph 7) whether the risk benefit ratio needs to be re-assessed and will inform the Trial Clinical Monitor (TCM) accordingly. A fundamental consideration is the safety of those who would be at potential risk due to their participation in the trials. The administrative organisation of the DSMB meetings is the responsibility of the sponsor (or nominated representative). The provision of the statistical tables and data listings is the responsibility of the DSMB 'programmer', in this trial also the Chair.

The responsibilities of the DSMB are:

- to ensure that patient safety is maintained by monitoring the trial for possible harmful effects of the test treatments
- to evaluate accrued data in order to recommend whether the study should continue, be modified or stopped for safety concerns or ethical reasons;
- to provide the sponsor with advice about the conduct of the trial and the integrity of the data, so as to protect the validity and scientific integrity of the trial.

The DSMB shall operate under this DSMB Charter.

The DSMB is an independent multidisciplinary group of 9 members selected for their relevant expertise on SCD and/or their experience in clinical trials. They will have agreed to the contents of this document and be aware of the responsibilities inherent in the operation of this DSMB. The DSMB will include experts, that collectively, have experience in the management of pediatric patients with SCD and also include a biostatistician knowledgeable in statistical methods used in clinical trials and in DSMB.
After the members of the DSMB confirm their participation in the DSMB, an initial meeting will be organised either in person during the initiation of the trial or if needed via a telephone conference. At the initial meeting the DSMB members together with the sponsor agree to appoint one of members to act as a chairman of the DSMB. The responsibilities of the chairman are outlined in this document.
Stefan Suciu was appointed the independent Statistician (iSTAT) for the DSMB. The iSTAT is not involved in T-Haplo for SCD projects and not allowed to dissemble unblinded information to the study/project team. He is not a DSMB member and will not be able to vote when (in the Executive Closed Session of the DSMB) voting will take place. The iSTAT will present data to the DSMB in closed session and will report to the DSMB Chair.
Membership will last until the duration of trial conduct. Selection of DSMB members is the responsibility of the T-Haplo for SCD project team in conjunction with corporate drug safety and therapeutic area taking into account considerations discussed above as well as their availability for the duration of the study and their ability to attend the majority of meetings. This is particularly important for the Chairman of DSMB.
If a member leaves the DSMB, a replacement will be proposed by the T-Haplo for SCD project team, in conjunction with corporate drug safety and therapeutic area, and of course in touch with remaining members of the committee. If the chairman of the DSMB leaves, a new chairman will be selected again from all DSMB members in conjunction with corporate drug safety and therapeutic area.
DSMB members are free of major financial or intellectual conflicts of interest that could prevent them from objectively reviewing the interim data and giving advice to the Sponsor. Conflict of interest declarations are on file. All DSMB members are asked to sign a statement declaring the absence of any serious conflict of interest (*appendix A.5*).
The DSMB members will disclose to fellow members any consulting agreements or financial interests they have with the sponsor. The DSMB members will be responsible for advising fellow members of any changes in these consulting agreements or financial interests that occur during the course of the trial. If a conflict of interest for one of its members arises during the trial conduct, the DSMB Chairman will take appropriate action, e.g. either recommend that the member resign or consult further with the sponsor.

The DSMB will meet at regular intervals over the entire period that patients are being treated in the context of the T-Haplo-SCD Trial. For details on meeting frequency, cf. a*ppendix A.5*. The DSMB may elect to meet at additional time points in order to re-evaluate DSMB procedures and its recommendations during the course of the study, or to advise the T-Haplo for SCD project team regarding final data processing (e.g. if additional tables or listings are required). DSMB membership is for the duration of the trials, until the last patient has ended the treatment phase.

Full addresses of members are documented in the *appendix A.5* and the address of the independent statistician in documented in the *section 'adresses*' and in the *appendix A.1.*

9.5. Other ethical and regulatory issues

During the course of the study, the Sponsor is obliged to submit to the IEC the following:

- Substantial Amendments to the protocol
- Serious and unexpected AEs (SUSARs) and their outcomes
- Development safety update report.

If a significant safety issue is identified, either from an ICSR (individual case safety report) or from review of aggregate data, then the Sponsor will issue prompt notification to all parties involved, i.e. regulatory authorities, investigators and IECs.

A significant safety issue is one that has a significant impact on the course of the clinical study or program (including the potential for suspension of the study program or amendments to protocols) or warrants immediate update of informed consent.

9.6. Data quality assurance

The Sponsor will conduct a face-to-face initiation meeting for all centres that intend to join the trial. Additional sites, in particular international sites that will join later, will be initiated via webinars and other electronic means. In order to verify the qualifications of each investigator and site, spot inspections of sites can be conducted at the discretion of the Sponsor to inform the investigators of responsibilities and procedures for ensuring adequate and correct documentation. The investigators are required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each study participant. These records will be collected online via an eCRF. All information recorded in the eCRF for this study must be consistent with the patient's source documentation (i.e. medical records).

To ensure data quality and completeness the following have to be observed:

- Individual AE Reports fully documented in the eCRF
- Diligent follow-up of each case
- Retaining of investigator's verbatim AE terms (documenting any Sponsor differences)
- Consistent and accurate codification of reported terms.
- Consistent and accurate conduct of the clinical trial
- Source data verification (i.e. medical records)

The responsible person in the respective stem cell laboratories will be primarily responsible for the quality and safety of the cell product. The quality control and safety data will be subject to secondary post-hoc monitoring by the clinical monitor. Should the clinical monitor detect a violation of the quality control and product release standards, he/she will immediately inform the sponsor.

9.7. Case report forms and source documentation

All relevant data collected during the study for all of the patients enrolled in the study will be recorded in the eCRF. The data will be entered by the responsible investigator or a person authorized by him in a timely manner. The physician will confirm the completeness, correctness and plausibility of the data by his signature. All source documents from which eCRF entries are derived should be placed in the patient's medical records.

The original data in the eCRF for each patient will be checked against source documents at the study site by the clinical monitor. Additions and corrections will be dated and signed by the responsible physician or an authorized person. Reasons must be given for corrections that are not self-explanatory. All data are stored in a central database. Instances of uninterpretable data will be discussed with the investigator for resolution. If corrections or additions are needed after checking the eCRF, a corresponding query must be formulated and forwarded to the physician for his response.

9.8. Trial monitoring

The Sponsor will co-ordinate/delegate monitoring activities to ensure that the study is conducted according to the current protocol version, that the human rights, safety and well-being of study participants are protected, and that the integrity of trial data is verifiable. Trained personnel will monitor the conduct of the study.

Prior to patient recruitment, the trial centre will receive a site initiation visit, during which a Sponsor representative will review the protocol and the eCRF with centre staff and provide any necessary training. During the study, trial monitors will maintain contact with trial centre personnel to track the progress of the trial, respond to any problems, and provide general assistance and support.

Trial monitors will periodically visit the trial centre in person, by prior arrangement with the PI. These monitoring visits will enable monitors to exchange information between the Sponsor and PI, check protocol and regulatory compliance, verify the quality of trial data, and formally evaluate the competence

and ongoing suitability of the site. During monitoring visits, monitors will check the eCRF for completeness and plausibility as well as perform source data verification (SDV) on specified data items, as a routine quality control check. eCRF entries will be compared with the corresponding source documents to ensure that data are being reported in full and without error. Discrepancies detected during SDV will be raised as data queries and sent to the trial centre for resolution. SDV will be performed for the data items specified in the study-specific monitoring guidelines. Monitors will also verify that written informed consent has been obtained correctly from all trial participants, check adherence to trial eligibility criteria and focus on defined safety parameters.

A risk-based monitoring approach will be used.

Key trial centre personnel must be available to assist the monitor during a monitoring visit. It is the obligation of the PI to facilitate monitoring activities by ensuring the availability of eCRF data, source documents, the ISF, and sufficient time to discuss trial-related matters with the monitor. The monitor must relay to the Sponsor the outcomes of all monitoring visits by means of a formal, written report. Any problems relating to local procedures, facilities, graft accountability, technical equipment or medical staff should be highlighted in the report.

If serious and/or persistent discrepancies are uncovered during monitoring activities, the Sponsor should be notified by the monitor at the earliest opportunity and reserve the right to sanction additional follow-up investigations.

The appropriate level of monitoring will be reviewed by the Sponsor on an ongoing basis and modified accordingly.

The trial centre may be suspended from recruitment in the event of severe and/or persistent failure to meet the expected standards.

9.9. Access to source data

The PI must retain source data for each trial subject and all information in the eCRFs must be traceable to these source documents. The duty of the clinical monitor is to review protocol compliance, compare eCRF and individual patient's medical records, assess graft accountability and ensure that the study is being conducted according to pertinent regulatory requirements. eCRF entries will be verified with source documentation.

Regulatory authorities, IECs and other authorized persons (e.g. CRAs monitoring the study) may wish to carry out source data checks or on-site audits/inspections. Direct access to all study-relevant records and relevant source documents will be required for these inspections and audits. They will be performed giving due consideration to data protection and medical confidentiality. The investigator agrees to ensure all necessary support at all times.

9.10. Data processing

All data will be entered in a central data base. The data review and data handling document has to be developed during the initiation phase of the study. It will include specifications for consistency and plausibility checks on data and will also include data-handling rules for obvious data errors.

9.11. Archiving study records

According to ICH guidelines, essential documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product.

The essential documents of this study will be retained for a longer period if required by the applicable legal requirements. All relevant source documents have to be archieved according to §14 of the German Transfusion Law.

9.12. Publication policy

All information concerning this study and not previously published is considered confidential information. All study results remain property of the Sponsor.
The results of the clinical study will be published after complete data collection and evaluation. Prof. S. Corbacioglu, as the National Coordinating Investigator of the study, will be the senior author of the main publications. Principal investigators for pediatric patients and principal investigators for adult patients with the highest rate of recruited and completely documented patients will be the first author(s) of the main publication(s). All other principal investigators will be represented as authors of the publication according to the number of patients recruited and followed. Central study related collaborators such as laboratories, statistician, trial related team members will be particularly considered, if data analysed in these labs and by these institutions will be used within the publication. All further details will be regulated by the respective agreement between the investigator and the sponsor.

Any publication has to be approved of by the Coordinating Investigator and the Sponsor.

10. Accompanying projects

10.1. Chimerism, split-chimerism and immune reconstitution analyses

10.1.1. Background and rationale

Chimerism and split-chimerism analyses are of utmost importance since the assessment of the proportion of donor and recipient cells in the peripheral blood or bone marrow allows prediction of substantial complications such as rejection and disease reoccurrence after allogeneic HSCT (LIT). Classically, a mixed chimerism is treated with withdrawal of immunosuppression and donor lymphocyte infusions (DLI) to achieve full chimerism (LIT). However, the relevance of full chimerism in non-malignant diseases is less clear. According to our experience, in T-Haplo HSCT of non-malignant diseases both treatment options may be wrong and even harmful. In T-depleted HSCT, immaturity of developing donor T-cells need a paradoxical extension of immunosuppression rather than withdrawal and DLI can induce GVHD. Also, in HSCT of SCD, erythroid precursors appear to have an engraftment advantage reflected in a 'dissociated' split chimerism with a low level of T-cell but full erythroid engraftment which these patients present with. This situation does not need any intervention. Split-chimerism analyses assessing the proportion of donor and recipient cells in different cell subsets can therefore reveal a more detailed cellular constellation of donor and recipient and help with treatment decisions.

Chimerism tests are classically done via PCR-based STR analysis. However, this technique is labor-intensive and expensive, particularly when applied in split analysis as this requires upfront purification or sorting of the respective subpopulations.

In haploidentical HSCT, mismatched HLA-antigens can be used to distinguish donor and recipient cells. We therefore aim to develop and standardize FACS-based split chimerism analysis using a MACSQuant® flow cytometer on haplotype HLA antigens. The goal is to be able to offer this technique to the majority, if not all, haploidentically transplanted patients in this trial using also newly available directly or and secondarily fluorescence-labelled anti-HLA antibodies. Next to costs and labor, FACS based chimerism analysis will allow a faster, cheaper, widely available but also a more detailed analysis of hematopoietic subsets during reconstitution. To validate flow-based chimerism analysis, suitable patient samples will be centrally analyzed at defined time points in parallel via FACS and PCR technique.

Immune recovery is key for control of infectious complications and major HSCT-associated complications such as rejection and GVHD. These outcome parameters are predominantly relevant in T cell depleted haploidentical HSCT.

Therefore, the investigation and characterization of these immune cells in more detail by flow cytometry will provide important information on the reconstitution of relevant subpopulations post-HSCT and their influence on transplant related outcome.

During thymic development T cells mature to immunological effector cells but also gain self-tolerance. However, beginning early in life, the thymus undergoes a process of involution and is replaced by adipose tissue. Therefore, monitoring of the thymic function by measuring new T cell synthesis becomes a critical issue in immune reconstitution studies, in particular in haploidentical T cell depleted HSCT in adults. In addition to fluorescence-based assessment, T cell receptor excision circles (TRECs) measurement via PCR can be used as surrogate biomarkers for thymic activity and to assess novel T cell synthesis, a critical parameter with T cell depleted grafts.

10.1.2. Objectives

1. Before allogeneic-HSCT, HLA-phenotyping of the donor and recipient is performed in the participating centers. Based on these results we will validate potential HLA-antibodies in separate donor and recipient samples to detect mismatched alleles by flow cytometry analysis. In parallel samples need to be sent to the PCR-laboratory to establish PCR testing.
2. We aim to investigate the chimerism and split chimerism in peripheral blood (and bone marrow) of SCD patients with a HLA-mismatched donor at defined time points after HSCT comparing PCR and flow-based techniques. Peripheral blood samples will be analysed after leukocyte engraftment (WBC>1000/µl), on day 60, day 100, day 180 and day 360 while a BM sample will be obtained on day 100 and in case of unclear mixed chimerism status of the patient.
3. In patients with mixed chimerism results (<90% donor chimerism) we intend to analyse additional on day 80, day 240 with an additional BM sample on day 240 after HSCT.
4. In addition, we intend to correlate split chimerism results obtain in monocytes and thrombocytes with erythroid chimerism results to assess the possibility to monitor erythroid engraftment and recovery by surrogate parameters in the peripheral blood.
5. We will correlate chimerism data with clinical information such as rejection, engraftment, occurrence of GVHD, infectious complications, immunosuppressive treatment etc. in order to assess the predictive value.
6. As T cells are critically involved in major complications of HSCT such as rejection and GVHD, we seek to investigate the recovery of these cells and other immune cells in more detail by flow cytometry. By the early teens, the thymus begins to decrease in size and activity and is gradually replaced by adipose tissue. Thymic function can be studied by T cell receptor excision circles (TRECs), which are small circles of DNA created in T cells during their passage through the thymus as they rearrange their TCR genes. Their presence indicates maturation of T cells. These small DNA pieces can be detected by PCR technique.

10.1.3. Project plan

Before enrollment high resolution HLA-typing of the donor and recipient will be performed on a routine basis at the patient's treatment center.

To conduct these studies, HLA-typing results as well as peripheral blood and / or bone marrow samples of the recipient and his haploidentical donor will need to be obtained and transferred to our center before the start of treatment.

In a first step we aim to select and validate available fluorescent-labelled anti-HLA antibodies according to the HLA-typing results, which allow the detection of HLA disparities in donor and recipient samples by flow cytometry. Indirect staining using secondary labelled antibodies may be required in the absence of directly labelled antibodies. To assess chimerism in subpopulations additional, lineage-specific antibodies such as CD3 to detect T cells, CD14 to detect monocytes, CD56 to detect NK cells, CD19 to detect B cells will be applied. For determination of erythroid progenitor populations, a combination of CD71, CD117, CD105, CD34 and for thrombocytes CD41/CD61 will be used in flow cytometry analyses. In parallel subpopulations of T cells, B cells, monocytes and erythroid precursors will be purified by FACS-sorting using CD3, CD19, CD14 and CD235a and then submitted to routinely performed PCR-based chimerism analysis at the same time points. In an estimated proportion of about 30% of the

patients, mixed chimerism with an overall proportion of <90% donor cells is expected on day +60. This will enable to detect the proportion of donor and recipient cells in certain cell subsets and track changes over time. In addition, the comparison of results obtained by the different methodologies will allow to validate flow cytometry results from peripheral blood and bone marrow specimen for future use as diagnostic tool.

Flow cytometry analysis will also allow to characterize certain subpopulations of immune cells such as T cells, B cells and NK cells in more detail by assessing markers important for their activation, inhibition and function on their surface. In particular $CD3^+CD56^+$, $CD3^+TCR\alpha\beta$, $CD3^+TCR\gamma\delta$ T cells, naive $CD4^+TCR\alpha\beta$, memory $CD4^+TCR\alpha/\beta^+$, naive $CD8^+TCR\alpha\beta$, memory $CD8^+TCR\alpha\beta$, DN $TCR\alpha\beta$, TCR $Vd2^+TCR\gamma\delta$, TCR $Vd2^-TCR\gamma\delta$, naive Tregs, 'memory' Tregs and thymic output markers such as CD31 on T cells are of interest. Furthermore, detection of TRECs in the peripheral blood of SCD patients before and after HSCT via PCR will allow to study thymic function and its impact on T cell maturation as well as the effect on major complications such as rejection, graft failure or GvHD mediated by T cells in pediatric and adult patients.

10.1.4. Required patient samples

During routine blood drawings in the context of diagnostic work-up **prior to HSCT**, peripheral blood (18 ml) collected in EDTA tubes will be obtained from the recipient and his donor. Additional peripheral blood samples (18 ml) will be collected again in EDTA tubes 9 ml on **day +60, +100, +180, +360 post HSCT** during routine follow up visits. Bone marrow aspirates (15-20 ml) might also be needed to be collected in EDTA tubes on day +100 after HSCT. These samples will be used to assess immune reconstitution and PCR/flow chimerism as outlined in *appendix B.13*.

In patients with mixed chimerism, additional blood samples (18 ml) need to be obtained on day +80 and +240, bone marrow aspirates (15-20 ml) on day +240. Additional analyses including bone marrow are at the discretion of the trial coordinator. Samples will be collected at the indicated time points (+/- 5 days). For analysis of split chimerism, EDTA blood and bone marrow samples should be directly shipped at room temperature to Regensburg via overnight express, delivery Monday until Thursday (see *appendix B.9* for flow chart and *appendix B.13* for shipment information).

10.2. Fertility assessment

With the advancement of HSCT to the point of very low transplant-related mortalities, the question of fertility preservation is increasingly important, in particular for patients with non-malignant diseases. Therefore, we will explore prospectively all possible pre-transplant procedures for fertility preservation and use standardized protocols for assessment of puberty and fertility post-HSCT.

The risk of impaired fertility following HSCT is high (>80%), especially in patients undergoing HSCT during childhood and adolescence, and in those exposed to TBI, high-dose cyclophosphamide (> 19g/m^2 BSA), or melphalan/busulfan [102, 103]. The use of a Treosulfan based conditioning regimen demonstrated in a very small series of patients advantages over busulfan [104] and overall the used conditioning regimen (Flu-Treo-TT) is expected to cause less HSCT-related adverse effects, but the potentially beneficial impact of such a regimen on short and long-term gonadal function as well as on reproductive outcomes in adulthood has not been systematically evaluated yet. Therefore, it is recommended to offer fertility preservation (FP) counselling as an integral part of the pre-HSCT workup to all SCD patients undergoing HSCT in this trial. Fertility counselling should be offered by a dedicated and well-trained team, including a transplant physician as well as a fertility FP specialist [105]. *Table 4* provides a detailed program for fertility work-up and documentation of any FP procedure performed before HSCT. Together with standardized, long-term follow-up care focusing on gonadal function and reproductive outcomes (*Table 3*), these data will allow to evaluate the impact of the proposed reduced-intensity conditioning regimen on the risk of gonadal failure and reproductive outcomes.

The current practice of FP counselling and performance of FP procedures differ in European countries, according to national recommendations, local logistics, technical experience, and reimbursement by

national insurance systems. Nevertheless, in the interest of the patients, FP procedures are strongly recommended.

Long-term daily oral hydroxyurea (HU) treatment has been shown to reduce or prevent many acute and chronic complications of SCD and therefore most of the patients will be on HU prior to HSCT.
As HU acts as an antimetabolite, it may have adverse effects on spermatogenesis [106]. In female, available data on the impact of HU on fertility is limited. Nevertheless, a wash-out period of 3-6 months after the re-implantation of the ovarian tissue (before a planned pregnancy) should be considered, to reduce embryonic toxicity of HU released from the re-implanted tissue.
Due to the risk of SCD-related complications caused by pausing of HU therapy, it is not recommended to suspend HU prior to fertility preservation procedures.

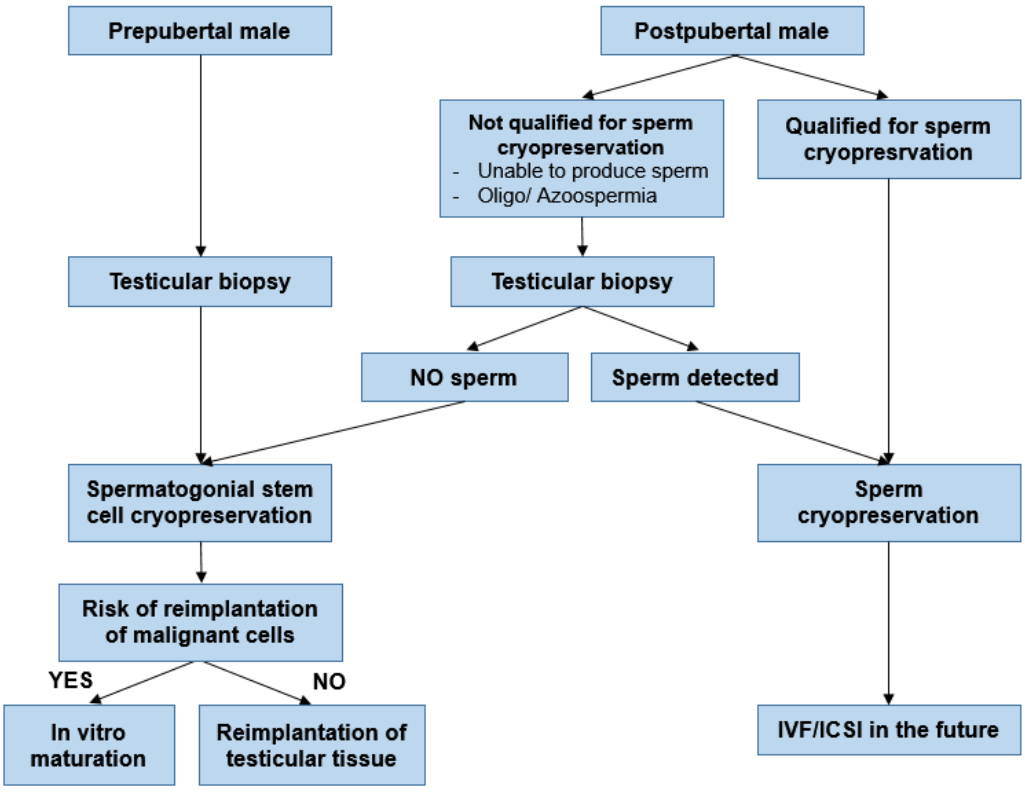
10.2.1. Specific issues

Ethical concerns may arise in the context of fertility preservation procedures, especially in paediatric patients. These may be related to the process of decision making (parents' conceptions and wishes vs. a child's current and presumed future attitudes towards FP), and more generally a child's or adolescent's ability to give consent to FP procedures. Other aspects concern the use of experimental FP methods in minors, religious aspects, and the disposition of stored tissue or gametes after a patient's death.

10.2.2. Fertility preservation options in male

For male patients, a variety of options exist for FP such as gonadal shielding, sperm cryopreservation, testicular sperm extraction (TESE), and testicular tissue cryopreservation (TTC). The decision, which method should be applied depends on the attained stage of pubertal development of the patient (see ***table 3*****,** FP options for male patients).

Table 3: Fertility preservation options in male patients



In postpubertal patients, the following established FP options can be proposed:

10.2.2.1. Sperm cryopreservation

Sperm cryopreservation is the most established option for FP in males [107]. In the majority of cases, sperm cryopreservation is simple to perform, safe, and not invasive. It should be recommended to all postpubertal boys and adult men who will receive gonadotoxic treatment. If a pubertal boy is unable to produce an ejaculate, alternative methods such as vibrator stimulation or electrostimulation under general anaesthesia may be offered [108]. The optimal time point for sperm cryopreservation is prior to any gonadotoxic treatment, as any exposure may severely impact successful sperm collection [109]. Furthermore, potential negative effects on DNA integrity shortly after exposure to gonadotoxic agents cannot be ruled out. Pubertal development is a better predictor for spermarche than age. Patients considered for sperm cryopreservation should at least have attained Tanner stage 3 and a testis volume >5 ml [105, 110]. Pregnancy rates achieved using cryopreserved sperm samples range from 23% to 57% [110]. The rate of fetal abnormalities and perinatal outcomes are similar to the general population [111]. It is important to inform patients, that sperm quality may be impaired even before gonadotoxic treatment due to the underlying chronic disease and/or HU treatment.

10.2.2.2. Testicular sperm extraction (TESE)

Testicular sperm extraction can be offered to patients with azoospermia, peripubertal patients with anejaculation, and failure or rejection of neurostimulatory methods. Spermatozoa are extracted directly from testicular tissue after a microsurgical procedure under anaesthesia. The procedure has an overall favourable safety profile with a low incidence of complications restricted mostly to scrotal hematoma, infections, pain, and swelling. Testicular damage with permanent impairment of gonadal function or loss of a testes following operation for TESE is a very rare event [112]. As for sperm cryopreservation, patients considered for TESE should have attained Tanner stage 3 and a testis volume >5 ml[105]

In prepubertal boys, the following experimental FP options may be offered:

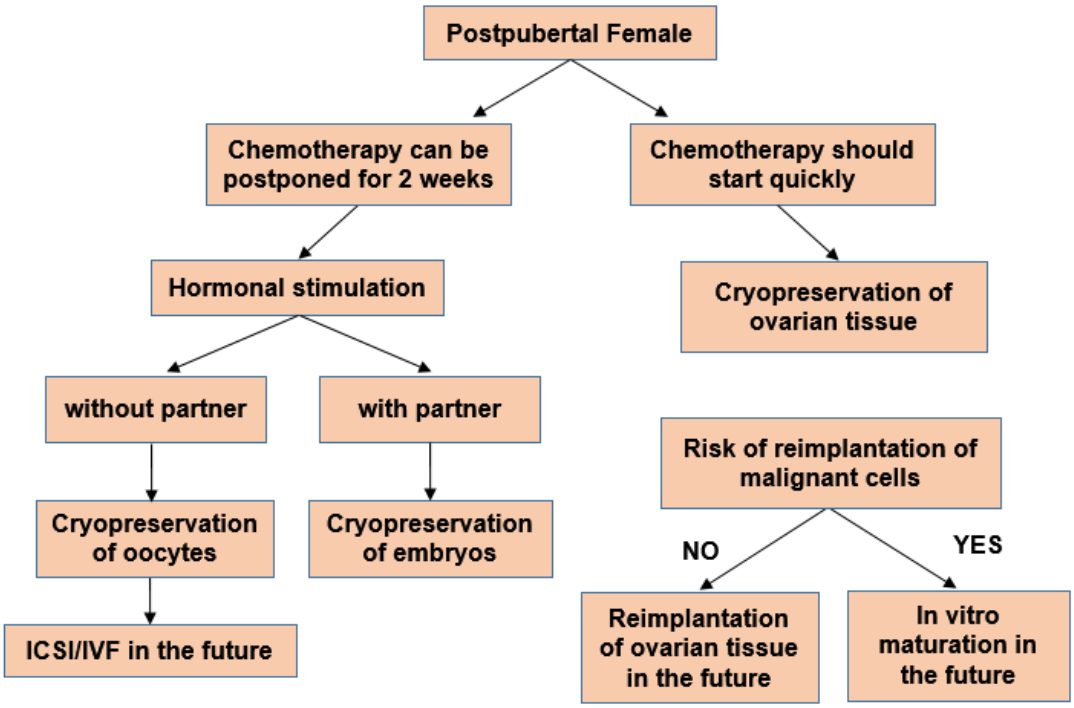
10.2.2.3. Testicular tissue cryopreservation

In prepubertal boys, fertility preservations options are limited due to the lack of mature spermatozoa. A promising, but currently experimental FP method is testicular tissue cryopreservation (TTC). TTC involves the harvesting of testicular tissue after a trans-scrotal biopsy and subsequent cryopreservation. TTC is well tolerated, with minimal postoperative morbidity. To date, no human pregnancy following prepubertal TTC has been reported. TTC relies on the future development of techniques for maturation of spermatogonial stem cells into mature sperms, or re-transplantation of the harvested tissue with into the testis. In animal models, promising results have been published with autologous grafting of cryopreserved prepubertal rhesus testis producing sperm and offspring [109]. Additional research is needed to optimize protocols and techniques. Cases of prepubertal patients considered for TTC should be discussed individually by an institutional review board. Furthermore, due to the currently experimental character of TTC in prepubertal boys and the importance of obtaining valid fertility outcome data in this population, we recommend against performing TTC in prepubertal boys outside of IRB-approved study protocols.

10.2.3. Fertility preservation options in female

Female fertility preservation options include mature oocyte cryopreservation and ovarian cortex cryopreservation, depending on the pubertal status of the patient (see see ***table 4***, fertility preservation options in female patients).

Table 4: Fertility preservation options in female patients



10.2.3.1. Prepubertal females

Cryopreservation of ovarian tissue for potential later maturation, gamete production or reimplantation in the pelvic cavity is the only option for FP in prepubertal girls. These procedures must still be classified as experimental, although there are emerging case reports of live births after re-transplantation of ovarian tissue harvested in patients before menarche [113-115].

10.2.3.2. Postpubertal females

Oocyte cryopreservation: Transvaginal oocyte collection after hormonal ovarian hyperstimulation, followed by cryopreservation is an established technique in adult females and postpubertal girls. Ooocyte cryopreservation and gonadotropin administration can cause severe ovarian hyperstimulation syndrome. There are some case reports in patients with SCD, reporting severe pain crisis following gonadotropin administration [116], but these observations have not yet led to a systematic evaluation of a potential risk increase.

On the basis of an elevated risk of sickle cell specific complications (e.g. cerebral insult, acute thorax syndrome) associated with gonadotropin administration or ovarian hyperstimulation syndrome in SCD patients, we advise to strictly avoid ovarian hyperstimulation in female SCD patients and recommend performing ovarian tissue cryopreservation also in postpubertal patients [117].

General considerations:

It appears to be safe to schedule cryopreservation surgery at least 1–2 weeks before beginning a conditioning regimen and transplant procedure. An ovary or parts of an ovary have to be removed surgically, often by laparoscopy.

It is advisable to combine the surgical interventions i.e. for central line insertion under one anaesthesia after the exchange transfusion in order to avoid SCD related crisis.

10.3. Pharmacokinetics and pharmacodynamics of Grafalon®

Aim is to establish an evidence-based dosing regimen of Grafalon® (ATG Neovii) using pharmacokinetic and pharmacodynamic studies to understand inter-individual variability to optimize dosing and reach an improved clinical outcome in patients treated with HSCT.
The lead product of NEOVII, anti-thymocyte globulin (ATG), Grafalon has approval for use in immunosuppressive therapies for prevention of graft-versus-host disease (GVHD) in hematopoietic cell transplantation (HCT), rejection of transplanted solid organs and treatment of aplastic anemia. Grafalon is generated by harvesting and purifying IgG immunoglobulin from rabbits sensitized with human Jurkat cells (immortalized T cell line derived from a patient with T cell leukemia).
The Boelens-Nierkens research group of the UMC Utrecht has generated an evidence-based dosing regimen of a related ATG product, Thymoglobulin (Sanofi), and exposed the delicate balance between exposure to T cell-binding ATG (active ATG) and outcomes in children and adults. The pharmacokinetic model showed that clearance and volume of distribution are dependent on body weight in a non-linear manner [118]. It was observed in children that the chance of successful immune reconstitution (IR) decreased when the ATG exposure after HCT increased, especially in cord blood transplanted patients. Successful IR as associated with increased overall survival, caused by reduced non-relapse mortality and relapse-related mortality in myeloid leukemia. An optimal exposure before transplant also resulted in a lower incidence of aGVHD and GF [119-121]
Survival in adult HCT patients is also affected by exposure to ATG: an optimal exposure after transplant (60-95 AU ATG/day/mL) resulted in higher overall and event-free survival changes. Exposure above optimum led to higher relapse-related mortality and exposure below optimum led to increased non-relapse mortality [122-124].
Based on these studies (inter)national guidelines are reconsidered and an increasing number of centers will be dosing Thymoglobulin based on the weight of the patient and the absolute lymphocyte count before transplant. For high-risk patients, we in Utrecht perform online therapeutic drug monitoring to ensure adequate disease control, engraftment and immune reconstitution without increasing the chances of GVHD and/or relapses.

As the cell source for sensitization of rabbits is different, the polyclonal immunoglobulin composition is different between Thymoglobulin and Grafalon. This will possibly result in slightly different mechanism of action and clearance rate between these products. Given the dramatic effects of Thymoglobulin exposure on IR and associated clinical outcomes, the optimal exposure in patients using Grafalon should be determined as fast as possible to provide optimal survival chances.

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